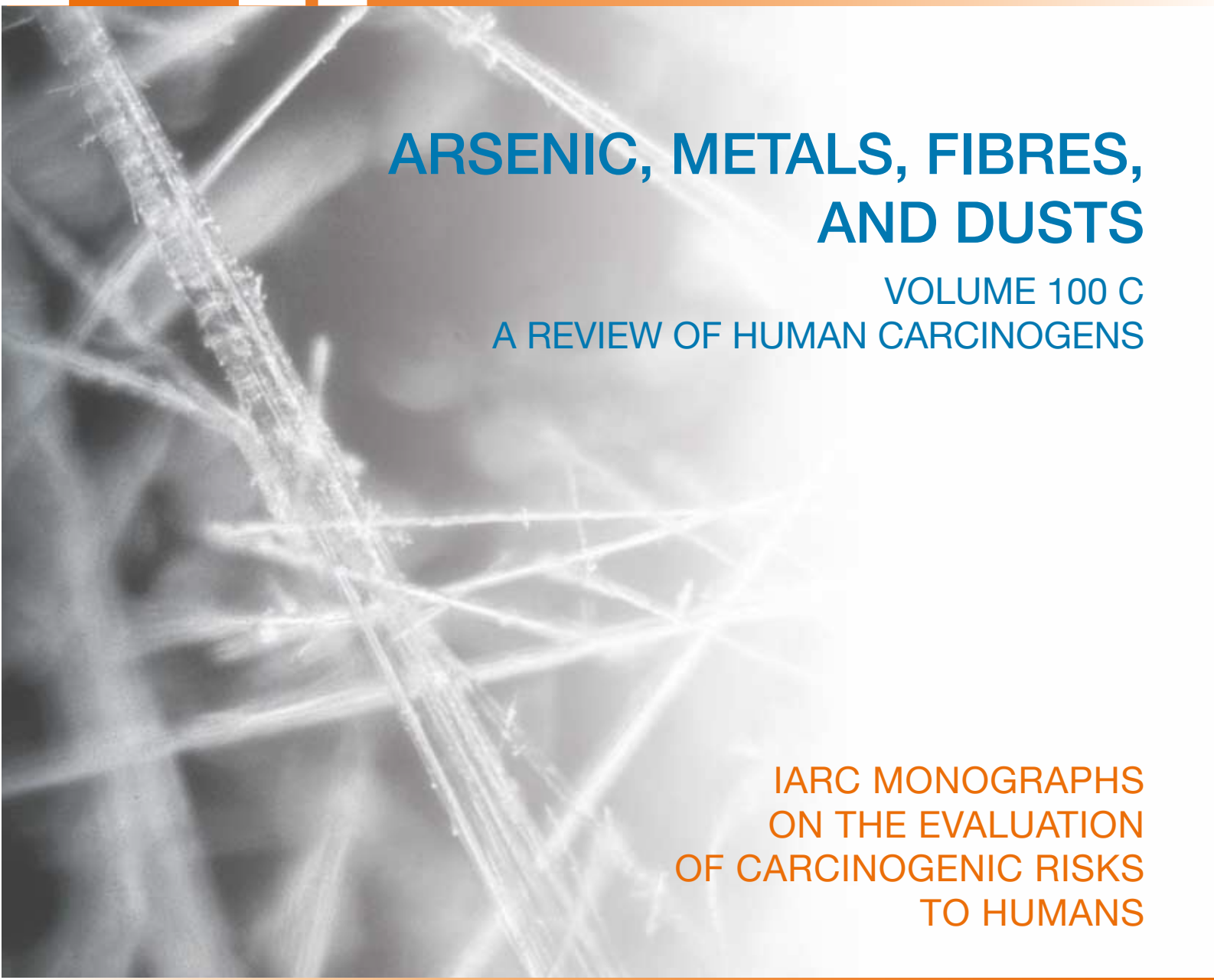
Exhibit 80

IARC MONOGRAPHS



ARSENIC, METALS, FIBRES, AND DUSTS

VOLUME 100 C
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

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physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental

animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is probably carcinogenic to humans.

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is possibly carcinogenic to humans.

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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CHROMIUM (VI) COMPOUNDS

Chromium (VI) compounds were considered by previous IARC Working Groups in 1972, 1979, 1982, 1987, and 1989 (IARC, 1973, 1979, 1980, 1982, 1987, 1990). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

Synonyms, trade names, and molecular formulae for selected chromium (VI) compounds are presented in Table 1.1. This list is not exhaustive, nor does it necessarily reflect the commercial importance of the various chromium-containing substances. Rather, it is indicative of the range of chromium (VI) compounds available.

1.2 Chemical and physical properties of the agents

Chromium (VI), also known as hexavalent chromium, is the second most stable oxidation state of chromium. Rarely occurring naturally, most chromium (VI) compounds are manufactured (products or by-products). Chromium (VI) can be reduced to the more stable chromium (III) in the presence of reducing agents (e.g. iron) or oxidizable organic matter (OSHA, 2006). Selected chemical and physical properties of various chromium (VI) compounds are presented in the previous *IARC Monograph* (IARC, 1990).

Chromium (VI) compounds are customarily classed as soluble or insoluble in water. Examples of water-soluble chromium (VI) compounds are sodium chromate (873 g/L at 30 °C) and potassium chromate (629 g/L at 20 °C). Water-insoluble chromium (VI) compounds include barium chromate (2.6 mg/L at 20 °C), and lead chromate (0.17 mg/L at 20 °C) (Lide, 2008). Compounds with solubilities in the middle of this range are not easily classified, and technical-grade compounds, such as the various zinc chromates, can have a wide range of solubilities (IARC, 1990). In the United States of America, the Occupational Safety and Health Administration (OSHA) has divided chromium (VI) compounds and mixtures into the following three categories: water-insoluble (solubility < 0.01 g/L), slightly soluble (solubility 0.01 g/L–500 g/L), and, highly water-soluble (solubility ≥ 500 g/L) (OSHA, 2006).

Chromium (VI) compounds are mostly lemon-yellow to orange to dark red in colour. They are typically solid (i.e. crystalline, granular, or powdery) although one compound (chromyl chloride) is a dark red liquid that decomposes into chromate ion and hydrochloric acid in water (OSHA, 2006).

Table 1.1 Chemical names (CAS names are given in italics), synonyms, and molecular formulae of selected chromium (VI) compounds

Chemical name	CAS No.[a]	Synonyms	Formula[b]
Ammonium chromate	7788-98-9	Chromic acid, ammonium salt; *chromic acid (H_2CrO_4), diammonium salt*; diammonium chromate	$(NH_4)_2CrO_4$
Ammonium dichromate	7789-09-5	Ammonium bichromate; ammonium chromate; *chromic acid ($H_2Cr_2O_7$), diammonium salt*; diammonium dichromate; dichromic acid, diammonium salt	$(NH_4)_2Cr_2O_7$
Barium chromate	10294-40-3 (12000-34-9; 12 231-18-4)	Barium chromate (VI); barium chromate (1:1); barium chromate oxide; *chromic acid (H_2CrO_4), barium salt (1:1)*	$BaCrO_4$
Basic lead chromate	1344-38-3 (54692-53-4)	C.I. 77 601; *C.I. Pigment Orange 21*; C.I. Pigment Red; lead chromate oxide	$PbO.PbCrO_4$
Calcium chromate	13765-19-0	Calcium chromium oxide; calcium monochromate; *chromic acid (H_2CrO_4), calcium salt (1:1)*; C.I. 77223; C.I. Pigment Yellow 33	$CaCrO_4$
Chromium [VI] chloride	14986-48-2	Chromium hexachloride; *(OC-6–11)-chromium chloride ($CrCl_6$)*	$CrCl_6$
Chromium trioxide	1333-82-0 (12324-05-9; 12324-08-2)	Chromia; chromic acid; chromic (VI) acid; chromic acid, solid; chromic anhydride; chromic trioxide; *chromium oxide (CrO3)*; chromium (VI) oxide; chromium (6+) trioxide; monochromium trioxide	CrO_3
Chromyl chloride	14977-61-8	Chlorochromic anhydride; chromium chloride oxide; chromium dichloride dioxide; *chromium, dichlorodioxo-(T-4)*; chromium dioxide dichloride; chromium dioxychloride; chromium oxychloride; dichlorodioxochromium	CrO_2Cl_2
Lead chromate	7758-97-6 (8049-64-7) 1344-37-2	*Chromic acid (H_2CrO_4), lead (2+) salt (1:1)*; C.I. 77600; C.I. Pigment Yellow 34; Chrome Yellow; lead chromate/lead sulfate mixture	$PbCrO_4$
Molybdenum orange	12656-85-8	*C.I. Pigment Red 104*; lead chromate molybdate sulfate red	$PbMoO_4$ $PbCrO_4$ $PbSO_4$
Potassium chromate	7789-00-6	Bipotassium chromate; *chromic acid (H_2CrO_4), dipotassium salt*; dipotassium chromate; dipotassium monochromate; neutral potassium chromate; potassium chromate (VI)	K_2CrO_4
Potassium dichromate	7778-50-9	*Chromic acid ($H_2Cr_2O_7$), dipotassium salt*; dichromic acid, dipotassium salt; dipotassium bichromate; dipotassium dichromate; potassium bichromate; potassium dichromate (VI)	$K_2Cr_2O_7$
Sodium chromate	7775-11-3	*Chromic acid (H_2CrO_4), disodium salt*; chromium disodium oxide; chromium sodium oxide; disodium chromate; neutral sodium chromate; sodium chromium oxide	Na_2CrO_4

Table 1.1 (continued)

Chemical name	CAS No.[a]	Synonyms	Formula[b]
Sodium dichromate	10588-01-9 (12018-32-5)	Bichromate of soda; *chromic acid ($H_2Cr_2O_7$), disodium salt*; chromium sodium oxide; dichromic acid, disodium salt; disodium dichromate; sodium bichromate; sodium dichromate (VI)	$Na_2Cr_2O_7$
Strontium chromate	7789-06-2 (54322-60-0)	*Chromic acid (H_2CrO_4), strontium salt (1:1)*; C.I. Pigment Yellow 32; strontium chromate (VI); strontium chromate (1:1)	$SrCrO_4$
Zinc chromate[c]	13530-65-9 (1308-13-0; 1328-67-2; 14675-41-3)	*Chromic acid (H_2CrO_4), zinc salt (1:1)*; chromium zinc oxide; zinc chromium oxide; zinc tetraoxychromate; zinc tetroxychromate	$ZnCrO_4$
Zinc chromate hydroxides	15930-94-6 (12206-12-1; 66516-58-3)	Basic zinc chromate; chromic acid (H_6CrO_6), zinc salt (1:2); chromic acid (H_4CrO_5), zinc salt (1:2), monohydrate; chromium zinc hydroxide oxide; zinc chromate hydroxide; zinc chromate (VI) hydroxide; *zinc chromate oxide ($Zn_2(CrO_4)O$), monohydrate*; zinc hydroxychromate; zinc tetrahydroxychromate; zinc yellow[d]	$Zn_2CrO_4(OH)_2$ and others
Zinc potassium chromates (hydroxides)	11103-86-9 (12527-08-1; 37809-34-0)	Basic zinc potassium chromate; chromic acid ($H_6Cr_2O_9$), potassium zinc salt (1:1:2); *potassium hydroxyoctaoxodizincate dichromate (1-)*; potassium zinc chromate hydroxide; zinc yellow[d]	$KZn_2(CrO_4)_2(OH)$ and others

[a] Replaced CAS Registry numbers are given in parentheses.
[b] Compounds with the same synonym or trade name can have different formulae.
[c] The term 'zinc chromate' is also used to refer to a wide range of commercial zinc and zinc potassium chromates.
[d] 'Zinc yellow' can refer to several zinc chromate pigments; it has the CAS No. 37300-23-5.

1.3 Use of the agents

Chromium (VI) compounds are used widely in applications that include: pigment for textile dyes (e.g. ammonium dichromate, potassium chromate, sodium chromate), as well as for paints, inks, and plastics (e.g. lead chromate, zinc chromate, barium chromate, calcium chromate, potassium dichromate, sodium chromate); corrosion inhibitors (chromic trioxide, zinc chromate, barium chromate, calcium chromate, sodium chromate, strontium chromate); wood preservatives (chromium trioxide); metal finishing and chrome plating (chromium trioxide, strontium chromate), and leather tanning (ammonium dichromate). Chromium (VI) may be present as an impurity in Portland cement, and it can be generated and given off during casting, welding, and cutting operations (for example, of stainless steel), even if it was not originally present in its hexavalent state (NTP, 2005; OHCOW, 2005; OSHA, 2006).

1.4 Environmental occurrence

Chromium (VI) can occur naturally in the earth's crust, although it is primarily emitted to the environment as a result of anthropogenic activities. The occurrence and distribution of chromium in the environment has been extensively reviewed (Mukherjee, 1998; Kotaś & Stasicka, 2000; Rowbotham *et al.*, 2000; Ellis *et al.*, 2002; Paustenbach *et al.*, 2003; Guertin *et al.*, 2004; Reinds *et al.*, 2006; Krystek & Ritsema, 2007).

1.4.1 Natural occurrence

Only lead chromate (as crocoite) and potassium dichromate (as lopezite) are known to occur in nature (IARC, 1990).

1.4.2 Air

Chromium (VI) is reported to account for approximately one third of the 2700–2900 tons of chromium emitted to the atmosphere annually in the USA (ATSDR, 2008a). Based on US data collected from 2106 monitoring stations during 1977–84, the arithmetic mean concentrations of total chromium in the ambient air (urban, suburban, and rural) were in the range of 0.005–0.525 μg/m^3 (ATSDR, 2000).

1.4.3 Water

The concentration of chromium in uncontaminated waters is extremely low (< 1 μg/L or < 0.02 μmol/L). Anthropogenic activities (e.g. electroplating, leather tanning) and leaching of wastewater (e.g. from sites such as landfills) may cause contamination of the drinking-water (EVM, 2002). Chromium (VI) has been identified in surface water (n = 32) and groundwater samples (n = 113) collected from 120 hazardous waste sites in the USA (ATSDR, 2000), and 38% of municipal sources of drinking-water in California, USA, reportedly have levels of chromium (VI) greater than the detection limit of 1 μg/L (Sedman *et al.*, 2006).

1.4.4 Soil

Chromium is present in most soils in its trivalent form, although chromium (VI) can occur under oxidizing conditions (ATSDR, 2008a). In the USA, the geometric mean concentration of total chromium was 37.0 mg/kg (range, 1.0–2000 mg/kg) based on 1319 samples collected in coterminous soils (ATSDR, 2000).

1.4.5 Food

There is little information available on chromium (VI) in food. Most of the chromium ingested with food is chromium (III) (EVM, 2002).

1.4.6 Smoking

Tobacco smoke contains chromium (VI), and indoor air polluted by cigarette smoke can contain hundreds of times the amount of chromium (VI) found in outdoor air.

1.5 Human exposure

1.5.1 Exposure of the general population

The general population residing in the vicinity of anthropogenic sources of chromium (VI) may be exposed through inhalation of ambient air or ingestion of contaminated drinking-water (ATSDR, 2000).

1.5.2 Occupational exposure

Inhalation of dusts, mists or fumes, and dermal contact with chromium-containing products are the main routes of occupational exposure. Industries and processes in which exposure to chromium (VI) occurs include: production, use and welding of chromium-containing metals and alloys (e.g. stainless steels, high-chromium steels); electroplating; production and use of chromium-containing compounds, such as pigments, paints (e.g. application in the aerospace industry and removal in construction and maritime industries), catalysts, chromic acid, tanning agents, and pesticides (OSHA, 2006).

Occupational exposures to several specific chromium compounds are reported in the previous *IARC Monograph* (IARC, 1990). With respect to chromium (VI) compounds, the most important exposures have been to sodium, potassium, calcium, and ammonium chromates and dichromates during chromate production; to chromium trioxide during chrome plating; to insoluble chromates of zinc and lead during pigment production and spray painting; to water-soluble alkaline chromates during steel smelting and welding; and, to other chromates during cement production and use (see Table 10; IARC, 1990, and OHCOW, 2005) for lists of occupations potentially exposed to chromium (VI)).

Estimates of the number of workers potentially exposed to chromium (VI) compounds have been developed by CAREX (CARcinogen EXposure) in Europe. Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that 785692 workers were exposed to hexavalent chromium compounds in the European Union, with over 58% of workers employed in the following four industries: manufacture of fabricated metal products except machinery and equipment (n = 178329), manufacture of machinery except electrical (n = 114452), personal and household services (n = 85616), and manufacture of transport equipment (n = 82359). CAREX Canada (2011) estimates that 83000 Canadians are occupationally exposed to chromium (VI) compounds. Industries in which exposure occurred include: printing and support activities; architectural/structure metal manufacturing; agricultural, construction, mining machinery manufacturing; specialty trade contractors; boiler, tank, and container manufacturing; industrial machinery repair; auto repair; metalworking machinery manufacturing; steel product manufacturing; aluminum production; metal ore mining; coating, engraving, and heat treating. Welders were the largest occupational group exposed (n = 19100 men and 750 women).

Data on early occupational exposures to chromium (VI) are summarized in the previous *IARC Monograph* (IARC, 1990). Data from studies on chromium (VI) exposure published since the previous *IARC Monograph* are summarized below.

In a study to characterize occupational exposure to airborne particulate containing chromium, and to evaluate existing control technologies, the US National Institute for Occupational Safety and Health (NIOSH) conducted 21 field surveys during 1999–2001 in selected industries. Industries and operations

evaluated included: chromium electroplating facilities; welding in construction; metal cutting operations on chromium-containing materials in ship breaking; chromate-paint removal with abrasive blasting; atomized alloy-spray coating; foundry operations; printing; and the manufacture of refractory brick, coloured glass, prefabricated concrete products, and treated wood products. Personal breathing zone samples (full-shift and short-term) and general area samples were collected. Results were compared to the NIOSH recommended exposure limit (REL) of 1 μg/m^3 (for a 10-hour exposure). Full-shift personal exposures to chromium (VI) were in the range of 3.0–16 μg/m^3 at the electroplating facilities, and 2.4–55 μg/m^3 at a painting and coating facility that used products containing chromium (VI) (Blade *et al.*, 2007).

NIOSH conducted a health hazard evaluation of worker exposures during the welding and manufacturing of stainless steel products and fabricated piping systems. Personal breathing zone air sampling concentrations of chromium (VI) were above the NIOSH REL. The highest concentrations for nickel and chromium (VI) occurred during welding operations inside large stainless steel pipes (0.26 mg/m^3 and 0.36 mg/m^3), and while welding fins on a large stainless steel pipe (Hall *et al.*, 2005).

As part of an international epidemiological study of workers in the pulp and paper industry, Teschke *et al.* (1999) assembled and analysed 7293 previously unpublished exposure measurements collected in non-production departments from 147 mills in 11 countries. Chromium (VI) compounds were reported in 26 time-weighted average (TWA) samples from nine mills, with a mean airborne chromium (VI) concentration of 63 μg/m^3 (range, 0.04–1220 μg/m^3).

Proctor *et al.* (2003) analysed more than 800 measurements of airborne chromium (VI) from 23 surveys conducted during 1943–71 at a chromate production plant in Painesville, Ohio, USA. The highest chromium (VI) concentrations recorded at the plant occurred in shipping (e.g. bagging of dichromate), lime and ash, and filtering operations (maximum yearly TWA concentrations of 8.9, 2.7, and 2.3 mg/m^3, respectively). The data showed that concentrations in the indoor operating areas of the plant generally decreased over time, dropping from 0.72 mg/m^3 in the 1940s, to 0.27 mg/m^3 in 1957–64, and to 0.039 mg/m^3 in 1965–72.

In a study to assess industry compliance with existing and proposed standards, Lurie & Wolfe (2002) conducted a secondary data analysis of 813 chromium (VI) measurements collected in 1990–2000 by OSHA. Chromium (VI) was not detected in 436 measurements. In the remaining samples, the median 8-hour TWA concentration was 10 mg/m^3 (n = 197; range, 0.01–13960 mg/m^3), and the median ceiling concentration was 40.5 mg/m^3 (n = 180; range, 0.25–25000 mg/m^3). In the plating and polishing industry, the median 8-hour TWA concentration was 8.2 mg/m^3 (n = 65; range, 0.01–400 mg/m^3), and the median ceiling concentration was 23 mg/m^3 (n = 51; range, 1–410 mg/m^3).

Luippold *et al.* (2005) examined the mortality of two cohorts of chromate production workers constituting the current US chromium chemical industry, after engineering controls were implemented. Personal air monitoring sampling for chromium (VI) at the two plants resulted in approximately 5230 personal air-monitoring measurements taken during 1974–88 for Plant 1, and 1200 measurements taken during 1981–98 for Plant 2. Personal levels of chromium (VI) exposure were very low at both plants (geometric mean, < 1.5 μg/m^3 for most years; range of annual means, 0.36–4.36 μg/m^3). At both plants, the work areas with the highest average exposures were generally less than 10 μg/m^3 for most years.

In an occupational exposure study of chromium in an aircraft construction factory, personal airborne samples were collected in a group of 16 workers over a 4-hour period, and urinary samples were collected from 55

workers at the beginning of their work shift (on Monday), and at the beginning and end of their work shift (on Friday). The geometric mean air concentration was 0.17 μg/m^3 (GSD, 5.35 μg/m^3; range, 0.02–1.5 μg/m^3). Geometric mean creatinine levels were as follows: pre-shift Monday, 0.63 μg/g (GSD, 0.53 μg/g; range, 0.23–2.9 μg/g); pre-shift Friday, 0.95 μg/g (GSD, 0.94 μg/g; range, 0.25–4.8 μg/g); and post-shift Friday, 0.91 μg/g (GSD, 1.38 μg/g; range, 0.16–7.7 μg/g) (Gianello *et al.*, 1998).

2. Cancer in Humans

2.1 Introduction

A large number of case reports dating to the late 19th and early-to-mid-20th centuries raised suspicions that workers in various industries with exposure to chromium compounds, including chromate production, production of chromate pigments and chromium plating may be at risk of developing various cancers (Newman, 1890; Pfeil, 1935; Teleky, 1936; IARC, 1990). Beginning in the mid-20th century, cohort studies were undertaken in these industries as well as in some other occupations and industries with potential exposure to chromium compounds, such as ferrochromium or stainless steel production, welding, leather tanning, and some others. By the 1980s considerable evidence had accumulated on cancer risks of chromium-exposed workers, and leading to the identification of chromium (VI) compounds as a human carcinogen (IARC, 1990).

The strongest evidence presented at the time concerned the lung. There was weaker and less consistent evidence of effects on gastrointestinal sites, mainly stomach, and some reports of excess risks at several other organs, such as pancreas, prostate and bladder. Furthermore, there were some case reports and small clusters of nasal or sinonasal cavity cancers in workers exposed to chromium (VI). Based on the review of the previous *IARC Monograph*, and on a subsequent review of relevant epidemiological evidence accumulated since then, the Working Group focused the current review on those sites for which the evidence indicates possible associations with chromium (VI) compounds, namely: lung, nose, and nasal sinus. Because of recent controversy regarding possible effects on stomach cancer (Proctor *et al.*, 2002; Beaumont *et al.*, 2008), the Working Group also reviewed relevant evidence for this organ. For other organs, the number of reports of excess risks is unremarkable in the context of the numbers of studies that have been conducted, and thus they have not been reviewed.

There have been at least 50 epidemiological studies that could be informative about cancer risks related to chromium (VI). Many of the studies have given rise to multiple reports; sometimes these simply represent follow-up updates, but often the different reports also present different types of analyses of subgroups or of case–control analyses within a cohort. Only a minority of the studies contain documented measurements of chromium (VI) exposure, particularly measurements that pertain to the era of exposure of the workforce that was investigated. It was therefore necessary to select and present the evidence according to the availability of relevant exposure information. The studies were triaged into the following categories:

1. Cohort studies in industries in which workers were highly likely to have been exposed at relatively high levels. This included workers in chromate production, chromate pigment production, and chromium electroplating.
2. Cohort studies in which workers were possibly exposed to relatively high levels but not with the same degree of certainty or concentration as those in category a. This included stainless steel welders.
3. Other studies in which workers may have been exposed to chromium (VI), but with lower likelihood or lower frequency or lower

concentrations than workers in categories 1) and 2). Among the occupations/industries in this category were ferrochromium and stainless steel production, mild steel welding, general paint production, general spray painting, tanneries, gold mining, and nickel plating.

Studies in category 3) were not routinely included in the current review because there were sufficiently informative studies in categories 1) and 2), except if the authors presented information indicative of exposure to non-negligible levels of chromium (VI).

Most of the informative evidence comes from industry-based cohort studies, some of which have been complemented by nested case–control analyses. One of the main limitations of industry-based cohort studies is the usual absence of information on smoking and other potential confounders aside from age, sex, and race. Nonetheless, except for some case–control studies of nasal cancer, the Working Group relied on cohort studies to provide informative results.

For each study selected, the Working Group chose the most recent publication; occasionally there were results in earlier papers that were also deemed important to present here. Further, in each publication there are typically a large number of results presented by organ site, by demographic characteristics of workers, by some index of duration or dose of exposure, and sometimes by analysing the data in a nested case–control fashion. For the purposes of the current review, the Working Group selected the key results from each publication, typically including the most general result available for workers exposed to chromium (VI) as well as a result for a subgroup characterized by relatively high duration or dose of exposure, when there were enough numbers in such a category.

2.2 Cancer of the lung

Almost all of the relative risk estimates for cancer of the lung presented in Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.1.pdf) are greater than 1.0. Among chromate production workers, virtually all studies showed excess risks of lung cancer, except for a few estimates of risks for US workers hired since exposures were lowered (Luippold *et al.*, 2005), but these latter analyses had few subjects and low power.

Similarly, studies of chromate pigment production workers tended to show elevated risks of lung cancer in nearly all the cohorts and subcohorts reported, though not every relative risk estimate was statistically significant. Also, among chromium electroplating workers, there was a clear pattern of excess risks in most cohorts. Workers in other industries who may have had somewhat lower levels of chromium (VI) exposure than those in the previously mentioned industries, had a less convincing set of relative risk estimates, though nearly all were above 1.0.

A few of the cohort studies collected high-quality smoking histories, and incorporated these into nested case–control analyses; these tended to show elevated risks independent of smoking. Several other studies had collected partial or representative smoking frequencies among their workers, and for most of these studies, the main results were unlikely to have been meaningfully confounded by smoking patterns in the workers.

A recent meta-analysis estimated an overall standardized mortality ratio (SMR) of 1.41 (95%CI: 1.35–1.47) for lung cancer among 47 studies of workers with possible chromium (VI) exposure (Cole & Rodu, 2005). [The Working Group noted that because of the great difficulty in establishing equivalencies between different studies in terms of the types and levels of exposures to chromium (VI), the summary estimates are difficult to interpret. Further, it appears

that some of the study populations in that meta-analysis overlapped with each other.]

In aggregate, the results continue to show that exposure to chromium (VI) increases the risks of lung cancer.

Very few of the epidemiological studies provided results relating to specific chromium (VI) compounds. Workers in chromate production were likely to have been exposed to mixtures of sodium, potassium, calcium and ammonium chromates and dichromates; the highest and most consistent excess risks were observed in these cohorts. Workers in chromate pigment production and spray painting were likely to have been exposed to zinc and/or lead chromates, also resulting in high risks. Steel smelting and welding probably resulted in exposure to alkaline chromates, and risks reported in these cohorts tended to be less clear than among the chromate producers and the chromate pigment producers. Because there seemed to be increased risks in diverse industries involving exposure to a variety of chromium (VI) compounds of varying solubilities, this observation argues for a general carcinogenic effect of chromium (VI).

2.3 Cancer of the nose and nasal sinus

Cancer of the nose and nasal sinus is extremely rare, the incidence of which is roughly 1/100th of the incidence of cancer of the lung (Parkin *et al.*,1997). In fact, most cohorts of workers exposed to chromium (VI) do not report on of the incidence of nose and nasal sinus cancers. [The Working Group noted that this could mean there were none in the cohort or that the investigators did not examine and report it.]

Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.2.pdf) shows the nine (ten studies including Sorahan *et al.*, 1987) cohort studies that did report how many nasal cancers occurred. Combining those nine (ten) cohorts, there were mentions of 22 (25) cases of nasal or nasal sinus cancer. For the four cohorts that reported an expected as well as an observed number of cases, the aggregate was 12 observed and 1.5 expected giving an SMR of 8.0. Because several cohort studies failed to report any cases, it is difficult to integrate the appropriate observed and expected numbers from these studies into the overall estimate of risk from cohort studies. [The Working Group believed that many of the studies which made no report on nasal cancer actually had none.]

Case reports since the 1960s have reported 11 (12 including one case reported in Enterline, 1974) cases of nasal or nasal sinus cancer among chromate workers. Without any indication of person-years at risk, it is difficult to infer whether this represents an excess.

There have been three informative case–control studies on nasal and nasal sinus cancer. Two showed some indications of excess risk among workers with possible exposure to chromium (VI) compounds, but the study with the best exposure assessment protocol (Luce *et al.*, 1993) reported no excess risks for workers exposed to chromium (VI).

In aggregate, the epidemiological evidence remains suggestive but inconclusive regarding the effect of chromium (VI) on nasal and nasal sinus cancers. [The Working Group noted that systematic confounding by nickel exposure is unlikely in the cohorts presented in Table 2.2 online.]

2.4 Cancer of the stomach

There is little evidence of an association between exposure to chromium (VI) and cancer of the stomach; there are as many point estimates above 1.0 as there are below. There has been concern about possible hazards related to the ingestion of chromium (VI) in drinking-water, and one study in the People's Republic of China

(Zhang & Li, 1987) and a subsequent reanalysis of the Chinese data (Beaumont *et al.*, 2008) seem to indicate a somewhat elevated risk of stomach cancer in which drinking-water was heavily polluted by a ferrochromium plant. However, one single ecological study does not constitute rigorous evidence of an association between exposure to chromium (VI) and cancer of the stomach.

See Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.3.pdf.

2.5 Synthesis

The large majority of informative cohort studies indicate that there is an excess risk of lung cancer among workers exposed to chromium (VI), particularly in chromate production, chromate pigment production, and chromium electroplating. It is unlikely that any biases or chance can explain these findings.

There are some case reports, cohort studies and case–control studies that suggest a possible excess of cancer of the nose and nasal sinus among workers exposed to chromium (VI). However, this evidence is susceptible to publication and reporting biases because many of the cohort studies did not report on nasal cancers, and it is not clear how to evaluate the significance of the case reports.

There is little evidence that exposure to chromium (VI) causes stomach or other cancers.

3. Cancer in Experimental Animals

Chromium (VI) compounds have been tested for carcinogenicity by several routes in several animal species and strains (IARC, 1990), and the following paragraphs summarize some key findings from previous IARC evaluations of chromium (VI) compounds.

Calcium chromate induced lung tumours in mice (males and females combined) when given by inhalation (Nettesheim *et al.*, 1971) and local tumours when given by intramuscular administration (Payne, 1960). In rats it caused lung tumours (adenoma, squamous cell carcinoma, or adenocarcinoma) when given by intratracheal administration (Steinhoff *et al.*, 1986) or intrabronchial administration (Levy & Venitt, 1986), bronchial (carcinomas or squamous cell carcinomas) when administered by intrabronchial administration (Levy *et al.*, 1986), and local tumours in rats treated by intrapleural (Hueper, 1961; Hueper & Payne, 1962) or intramuscular administration (Hueper & Payne, 1959, 1962; Hueper, 1961; Roe & Carter, 1969).

Lead chromate (and its derived pigments), administered by subcutaneous injection (Maltoni, 1974, 1976; Maltoni *et al.*, 1982) or intramuscular injection cause malignant tumours at the site of injection and renal tumours (Furst *et al.*, 1976) in rats. Subcutaneous administration of basic lead chromate caused local sarcomas in rats (Maltoni, 1974, 1976; Maltoni *et al.*, 1982). In rats, zinc chromates caused bronchial carcinomas when administered by intrabronchial implantation (Levy *et al.*, 1986), and local tumours when given intrapleurally (Hueper, 1961), subcutaneously (Maltoni *et al.*, 1982) or intramuscularly (Hueper, 1961). Strontium chromate also caused bronchial carcinomas (intrabronchial implantation administration) (Levy *et al.*, 1986), and local sarcomas (intrapleural and intramuscular administration) in rats (Hueper, 1961).

Chromium trioxide when tested as a mist by inhalation caused nasal papillomas in mice (Adachi & Takemoto, 1987). Local tumours were observed in rats exposed to sintered chromium trioxide (Hueper & Payne, 1959). A low incidence of lung adenocarcinomas was induced after inhalation of chromium trioxide, and some lung tumours were observed in rats exposed by intrabronchial administration but neither were

statistically significant (Adachi *et al.*, 1986; Levy *et al.*, 1986; Levy & Venitt, 1986).

Sodium dichromate (when given by inhalation or intratracheal administration) caused lung tumours (benign and malignant) (Glaser *et al.*, 1986; Steinhoff *et al.*, 1986) in rats.

3.1 Studies published since the previous *IARC Monograph*

Since the previous *IARC Monograph* (IARC, 1990), studies in experimental animals have been conducted to evaluate oral exposure to chromium (VI). Table 3.1 summarizes the results of these studies, and the text below summarizes the major findings for each specific compound.

3.1.1 Sodium dichromate dihydrate

The National Toxicology Program (NTP) conducted 2-year drinking-water studies of sodium dichromate dihydrate in male and female B6C3F$_1$ mice, and in male and female F344 rats. In rats, sodium dichromate dihydrate significantly increased the incidence of squamous cell epithelium tumours of the oral mucosa or tongue in the high-dose groups (516 mg/L) of males and females. Trend analysis indicated a dose–response relationship in both males and females. In mice, sodium dichromate dihydrate significantly increased tumours (adenomas or carcinomas) of the small intestine (duodenum, jejunum, or ileum) in the two-highest dose groups of males (85.7 and 257.4 mg/L) and females (172 and 516 mg/L). Dose–response relationships were observed in both sexes (NTP, 2008).

3.1.2 Potassium chromate

Davidson *et al.* (2004) studied the effects of potassium chromate on ultraviolet(UV)-induced skin tumours in female hairless mice (CRL: SK1-hrBR). Mice were exposed to UV alone, various concentration of potassium chromate alone (given in the drinking-water), and UV together with various concentrations of potassium chromate. Administration of drinking-water containing potassium chromate did not induce skin tumours alone. However, chromate treatment significantly increased the multiplicity of UV-induced skin tumours, and the multiplicity of malignant UV-induced skin tumours. Similar results were found in male and female hairless mice (Uddin *et al.*, 2007). The analysis of skin indicated that UV treatment increased the level of chromium in the exposed skin (Davidson *et al.*, 2004).

3.2 Synthesis

The administration of calcium chromate in mice and sodium dichromate in rats by inhalation caused lung cancer. Calcium chromate and sodium dichromate administered by intratracheal instillation caused lung cancer in rats. Intratracheal administration of calcium chromate, zinc chromate, and strontium chromate caused lung cancer in rats. Several chromium compounds by repository injection (calcium chromate, lead chromate, zinc chromate, strontium chromate) caused local sarcomas. Oral administration of sodium dichromate to rats and mice caused cancer of the oral cavity and of the gastrointestinal tract. Potassium chromate given orally, although not given alone, enhanced UV-induced skin carcinogenesis, indicating tumour systemic effects.

Table 3.1 Studies of cancer in experimental animals exposed to chromium (VI) (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance[a]	Comments
Sodium dichromate dihydrate				
Rat, F344/N (M, F) 2 yr NTP (2008)	Drinking-water 0, 14.3, 57.3 172, 516 mg/L Average daily doses: M-0, 0.6, 2.2 6, 17 mg/kg bw F-0, 0.7, 2.7, 7, 20 mg/kg bw *ad libitum* 50/group/sex	Oral mucosa (squamous cell carcinomas):[b] M–0/50, 0/50, 0/49, 0/50, 6/49 (12%) F–0/50, 0/50, 0/50, 2/50 (4%), 11/50 (22%) Tongue (squamous cell papillomas or carcinomas): M–0, 1, 0, 0, 1 F–1, 1, 0, 1, 0 Oral mucosa or tongue:[c] M–0/50, 1/50 (2%), 0/49, 0/50, 7/49 (14%) F–1/50 (2%), 1/50 (2%), 0/50, 2/50 (4%), 11/50 (22%)	M: $P < 0.05$ (high dose); $P_{trend} < 0.001$ F: $P < 0.001$ (high dose); $P_{trend} < 0.001$ M: $P < 0.01$; $P_{trend} < 0.001$ F: $P < 0.01$ (high dose); $P_{trend} < 0.001$	Age at start, 6–7 wk 99.7% pure No treatment effects on survival Decreased bw in high-dose males and females Decreased water consumption of the 2 highest doses
Mouse, B6C3F$_1$ (M, F) 2 yr NTP(2008)	Drinking-water M: 0, 14.3, 28.6, 85.7, 257.4 mg/L F: 0, 14.3, 57.3, 172, 516 mg/L Average daily doses: M–0, 1.1, 2.6, 7, 17 mg/kg bw F–0, 1.1, 39.9, 9, 25 mg/kg bw *ad libitum* 50/group/sex	Small intestine (adenomas): M–1/50 (2%), 1/50 (2%), 1/50 (2%), 5/50 (10%), 17/50 (34%) F–0/50, 1/50 (2%), 2/50 (4%), 15/50 (30%), 16/50 (32%) Small intestine (carcinomas): M–0/50, 2/50 (4%), 1/50 (2%), 3/50 (6%), 5/50 (10%) F–1/50 (2%), 0/50, 2/50 (4%), 3/50 (6%), 7/50 (14%) Small intestine (adenomas or carcinomas):[d] M–1/50 (2%), 3/50 (6%), 2/50 (4%), 7/50 (14%), 20/50 (40%) F–1/50 (2%), 1/50 (2%), 4/50 (8%), 17/50 (34%), 22/50 (44%)	M: $P < 0.001$ (high dose); $P_{trend} < 0.001$ F: $P < 0.001$ (2 highest doses); $P_{trend} < 0.001$ M: $P < 0.05$ (high dose); $P_{trend} < 0.05$ F: $P < 0.05$ (high dose); $P_{trend} < 0.001$ M: $P < 0.001$ (high dose), $P < 0.05$ (85.7 mg/L), $P_{trend} < 0.001$ F: $P < 0.001$ (2 highest doses 172 and 516 mg); $P_{trend} < 0.001$	Age at start, 6–7 wk 99.7% pure No treatment effects on survival Decreased body weight in 2 highest female dose groups Decreased water consumption of the 2 highest doses (males and females) Most of the tumours were located in the duodenum

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance[a]	Comments
Potassium chromate (K_2CrO_4)				
Mouse, CRL: Sk1-hrBR (F) 224 d Davidson *et al.* (2004)	Group 1: Controls Group 2: UV only Group 3: 2.5 ppm K_2CrO_4 Group 4: 5 ppm K_2CrO_4 Group 5: UV +0.5 ppm K_2CrO_4 Group 6: UV + 2.5 ppm K_2CrO_4 Group 7: UV + 5 ppm K_2CrO_4: UV: 1 mo after K_2CrO_4 1.1 kJ/m^2 3 d/wk for 3 mo, followed by 1 wk break, and 1.3 kJ/m^2, 2 d/wk for 3 mo K_2CrO_4: 182 d, added to drinking-water every 7–10 d 120 animals	Skin (tumours): Groups 1, 3, 4–no tumours *Number of tumours (> 2mm/no of mice at 182 d):* Group 2–12/15 (0.8) Group 5–16/12 (1.39) Group 6–50/19 (2.63) Group 7–94/19 (5.02)	Group 6 vs Group 2, $P < 0.05$ Group 7 vs Group 2, $P < 0.01$	Age at start, 6 wk Chromium-only treatment had no effects on bw or toxicity Levels of chromium were measured in dorsal thoracic skin and abdominal skin in Groups 1, 4, and 7 UV + chromium had significantly higher chromium levels in back and underbelly skin

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance[a]	Comments
Mouse, CRL: Sk1-hrBR (M, F) 224 d Uddin *et al.* (2007)	Groups: treatment, *n* Group 1a: UV, 10 Group 1a: UV + 2.5 ppm K_2CrO_4, 10 Group 1c: UV + 5 ppm K_2CrO_4, 10 Group 2a: UV + 5 ppm K_2CrO_4, 10 Group 2b: UV + 5 ppm K_2CrO_4 + Vitamin E, 10 Group 2c: UV + 5 ppm K_2CrO_4 + selenium, 10 Mice administered K_2CrO_4 in drinking-water at 3 wk of age. 3 wk later UV treatment (1.0 kJ/m^2) 3 d/wk for 26 wk Vitamin E: 62.5 IU/kg Selenium: 5 mg/kg Group 1–males, Group 2–females (30/group)	Skin (number of tumours/mice at 26 wk): M– Group 1a: 1.9 ± 0.4 Group 1b: 5.9 ± 0.8 Group 1c: 8.6 ± 0.9 F– Group 2a: 3.9 ± 0.6 Group 2b: 3.5 ± 0.6 Group 2c: 3.6 ± 0.6	Group 1b vs 1a, $P < 0.001$ Group 1c vs 1a, $P < 0.0001$	Age, 3 wk Chromium had no effect on growth of the mice. Chromium levels in skin increased with dose Chromium also decreased the time until appearance of first tumours in males

[a] *P*-values for calculated by Poly 3- for NTP studies, which accounts for differential mortality in animals that do not reach terminal sacrifice.
[b] Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M: 0/300, F: 0/300.
[c] Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M: 2/300, range 0 to 2%; F: 3/300, range 0 to 2%.
[d] Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M:11/299, range 0–10%; F: 4/350, range 0 to 4%.
[e] Borneff *et al.* (1968) published in German.
[f] No information on tumour incidence of this group was reported by Sedman *et al.* (2006).
[g] Two-Tailed Fisher Exact Test; Authors stated significant but did not provide*P*-value.
[h] Untreated and chromium only, controls not included since no tumours were observed in the study by Davidson *et al.* (2004).
bw, body weight; d, day or days; F, female; M, male; mo, month or months; UV, ultraviolet; vs, versus; wk, week or weeks; yr, year or years

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans, the absorption, retention, and elimination of chromium compounds after exposure by inhalation depend on the solubility and particle size of the particular compound inhaled (for an extensive review, see ATSDR, 2008b). The retention may range from several hours to weeks. Inhaled chromium (VI) is readily absorbed from the respiratory tract. The degree of absorption depends on the physical and chemical properties of the particles (size, solubility), and the extent of reduction of the hexavalent form to chromium (III), which is absorbed to a much lesser extent. Thus, after intratracheal instillation in rats, 53–85% of chromium (VI) compounds with a particle size < 5 µm are absorbed into the bloodstream, with higher absorption rates in case of more soluble compounds; the rest remains in the lungs. For comparison, absorption of chromium (III) from the respiratory tract is only 5–30% (ATSDR, 2008b). The same factors mentioned above apply to absorption from the gastrointestinal tract, although absorption by this route is generally much less compared with that in the respiratory tract. Average absorption fractions determined in human volunteers for chromium (III) or chromium (VI) were reported as 0.13% or 6.9%, respectively. Chromium (VI) can penetrate human skin to some extent (ATSDR, 2008b).

In humans and rodents, absorbed chromium (VI) is distributed in nearly all tissues, with the highest concentrations found in the kidney, liver, and bone. Studies conducted by the NTP in male rats and female mice orally exposed to chromium (VI) for 2 years showed dose-related and time-dependent increases in total chromium concentrations in red cells, plasma, and in several organs. The total chromium content of the red cells was higher than that of plasma. The concentration of total chromium in the forestomach was found to be markedly higher in mice than in rats (NTP, 2008).

Within the human body, chromium (VI) undergoes a series of reduction steps to form the thermodynamically stable chromium (III). When reduction occurs extracellularly, this process can be considered as detoxification because the cell membrane is a nearly impermeable barrier for chromium (III). The remaining chromium (VI) is present as a mixture of chromate (CrO_4^{2-}) and hydrochromate ($HCrO_4^-$); because water-soluble chromates are iso-structural with sulfate and phosphate ions, they are readily taken up by sulfate channels. In case of poorly water-soluble chromates, particles of < 5 µm can be phagocytosed, and gradually dissolved intracellularly. Within the cell, chromium (VI) is reduced stepwise to chromium (III), giving rise to reactive intermediates as well as DNA and protein adducts. In blood, chromium (VI) is taken up into red blood cells, is reduced, and then bound to proteins. After exposure by inhalation, excretion occurs predominantly via the urine. Due to the low absorption of chromium compounds from the gastrointestinal tract, the major pathway of elimination after oral exposure is through the faeces (ATSDR, 2008b).

4.2 Genetic and related effects

The oxidation state of chromium is the most important factor when considering its biochemical activity (Beyersmann & Hartwig, 2008; Salnikow & Zhitkovich, 2008). Chromium (VI), but not chromium (III) compounds, have been shown to exert genotoxicity both *in vivo* and *in vitro*.

Lymphocytes of workers exposed to dusts of chromium (VI) compounds showed elevated frequencies of DNA strand breaks (Gambelunghe *et al.*, 2003), sister chromatid exchange (Wu *et al.*, 2001), and micronuclei (Vaglenov *et al.*, 1999; Benova *et al.*, 2002).

After intratracheal instillation in rats, chromium (VI) induced DNA strand breaks in lymphocytes (Gao *et al.*, 1992). After intraperitoneal injection of chromium (VI) to mice, micronuclei were induced in bone marrow. In contrast, no micronucleus induction was observed after oral administration, indicating that chromium (VI) does not reach the target cells to a high extent by this route of exposure (De Flora *et al.*, 2006). Chromium (VI) induces dominant lethal mutations in male mice (Paschin *et al.*, 1982).

In vitro, soluble chromium (VI) compounds are mutagenic in mammalian and bacterial test systems (De Flora *et al.*, 1990).

4.2.1 DNA damage

Chromium (VI) is unreactive towards DNA under physiological conditions. According to the uptake–reduction model originally established by Wetterhahn *et al.* (1989), chromium (VI) undergoes a series of reduction steps in cells, to form the thermodynamically stable chromium (III). Intracellular reduction does not require enzymatic steps but is mediated by direct electron transfer from ascorbate and non-protein thiols, such as glutathione and cysteine. During the reduction process, variable amounts of chromium (V) and chromium (IV) as well as organic radical species are generated; their exact nature, however, depends largely on the reducing species (Wetterhahn & Hamilton, 1989). Furthermore, comparative in-vivo and in-vitro studies revealed a major impact of the intracellular reductants on the nature and biological consequences of the resultant DNA lesions.

The major intracellular reductant under physiological conditions appears to be ascorbate, reaching millimolar concentrations in human tissues, and accounting for about 90% of chromium (VI) reduction reactions *in vivo* (Standeven *et al.*, 1992). In contrast, only micromolar concentrations of ascorbate are usually present in cell cultures (Quievryn *et al.*, 2002), which leads to an increase in thiol-mediated chromate reduction. When ascorbate is the reductant, two electrons are transferred, and chromium (IV) but not chromium (V) is generated as the first intermediate, whereas with cysteine as a reductant, predominantly chromium (V) is formed due to one-electron transfers (Stearns & Wetterhahn, 1994). In both cases, the final product is chromium (III), which reacts to produce different types of DNA lesions.

DNA lesions generated after exposure to chromium (VI) include chromium (III)–DNA adducts, DNA–protein and DNA–DNA interstrand crosslinks, DNA breaks as well as several oxidative DNA–base modifications. The predominant form of chromium (III)–DNA adducts are ternary adducts, where chromium forms a link between DNA and small molecules such as cysteine, histidine, glutathione or ascorbate, presumably arising from preformed chromium–ligand complexes during the reduction process. These adducts are formed primarily at phosphate groups, but the subsequent partial formation of chelates involving the phosphate group and the N^7-position of guanine have been suggested. Chelates formed from chromium–ascorbate particularly are potent premutagenic DNA lesions (Zhitkovich *et al.*, 2001).

The formation of DNA–protein crosslinks after chromate exposure is well established, but is estimated to account for less than 1% of chromium–DNA adducts. Biological consequences are likely to be disturbances of DNA replication and transcription. The formation of DNA–DNA crosslinks appears to be restricted to certain in-vitro conditions, due to severe steric hindrance upon intercalation of octahedral chromium (III) complexes (Zhitkovich, 2005).

DNA single-strand breaks may arise due to the reaction of chromium (V) with hydrogen peroxide, forming hydroxyl radicals. Nevertheless, if ascorbate is the predominant reductant under in-vivo conditions, the generation of chromium (V) and thus, single-strand

breaks, appears to be of minor importance (Quievryn *et al.*, 2003). Cytogenetic alterations in chromium (VI)-exposed cells in culture and *in vivo*, such as increased frequencies of chromosomal breaks and micronuclei, are suggested to be due to DNA double-strand breaks, produced by a cell-replication-dependent mechanism in the G2 phase of the cell cycle. Recent evidence suggests the involvement of mismatch repair in the formation of double-strand breaks. Thus, highly mutagenic ascorbate–chromium–DNA adducts lead to the error-prone repair of double-strand breaks through non-homologous end-joining. Furthermore, they induce mismatches during replication, leading to aberrant mismatch repair. Based on these findings, a model has been created to show that chronic exposure to toxic doses of chromium (VI) provokes the selective outgrowth of mismatch-repair-deficient clones with high rates of spontaneous mutagenesis, and thus, genomic instability (Reynolds *et al.*, 2007; Salnikow & Zhitkovich, 2008). In support of this model, chromium-induced cancers in exposed workers were associated with microsatellite instability and exhibited the loss of expression of MLH1, which is one of the essential mismatch-repair proteins (Takahashi *et al.*, 2005).

4.2.2 Oxidative stress

In the reduction of chromium (VI) to chromium (III) by cellular reductants, potentially toxic intermediates (oxygen radicals, sulfur radicals, and chromium radicals) are generated (Yao *et al.*, 2008). In a cell-free system, chromium (VI) reacted with glutathione to form chromium (V) and thiyl radicals (Wetterhahn *et al.*, 1989). Furthermore, after reduction of chromium (VI) by glutathione, chromium (V) can undergo Fenton-type reactions, producing hydroxyl radicals (Shi *et al.*, 1994), and 8-oxoguanine in isolated DNA (Faux *et al.*, 1992). In cultured mammalian cells, chromium (VI) induced the formation of superoxide and nitric oxide (Hassoun & Stohs, 1995). The administration of chromium (VI) to animals, which have higher tissue levels of ascorbate compared with cultured cells, did not induce the formation of 8-oxoguanine (Yuann *et al.*, 1999). This may be due to the lack of chromium (V) formation when ascorbate is the predominant reducing agent.

4.2.3 Further potentially relevant mechanisms

Besides direct genotoxic effects of chromium (VI) metabolites, chromate may activate various mitogen-activated protein kinases as well as transcription factors involved in inflammation and tumour growth. Nevertheless, because these effects have been observed in cell-culture systems and no distinct effects of chromium (VI) on cell proliferation have been shown, the relevance of these observations remains unclear at present. Perhaps of higher impact are the aneugenic properties of chromium (VI). Chronic treatment with lead-chromate particles induced neoplastic transformation of human bronchial cells, which was accompanied by centrosome amplification, and an increase in aneuploid metaphases (Xie *et al.*, 2007).

4.3 Synthesis

Several mechanisms are involved in the carcinogenesis induced by chromium (VI) that include the induction of DNA damage, the generation of oxidative stress and aneuploidy, leading to cell transformation. With respect to DNA damage, the spectrum of induced lesions appears to depend strongly on the cellular reductant involved. Thus, under physiological conditions with ascorbate as the major reductant, the generation of premutagenic ternary chromium–ascorbate–DNA adducts appears to be of major relevance, which may be linked to the increased number of mismatch-repair-resistant cells observed in chromate-induced lung tumours.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chromium (VI) compounds. Chromium (VI) compounds cause cancer of the lung. Also positive associations have been observed between exposure to Chromium (VI) compounds and cancer of the nose and nasal sinuses.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chromium (VI) compounds.

Chromium (VI) compounds are *carcinogenic to humans (Group 1)*.

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NICKEL AND NICKEL COMPOUNDS

Nickel and nickel compounds were considered by previous IARC Working Groups in 1972, 1975, 1979, 1982, 1987, and 1989 (IARC, 1973, 1976, 1979, 1982, 1987, 1990). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

Synonyms, trade names, and molecular formulae for nickel, nickel alloys, and selected nickel compounds are presented in Table 1.1. This list is not exhaustive, nor does it necessarily reflect the commercial importance of the various nickel-containing substances, but it is indicative of the range of nickel alloys and compounds available, including some compounds that are important commercially, and those that have been tested in biological systems. Several intermediary compounds occur in refineries that cannot be characterized, and are thus not listed.

1.2 Chemical and physical properties of the agents

Nickel (atomic number, 28; atomic weight, 58.69) is a metal, which belongs to group VIIIB of the periodic table. The most important oxidation state of nickel is +2, although the +3 and +4 oxidation states are also known (Tundermann *et al.*, 2005). Nickel resembles iron, cobalt, and copper in its chemical properties. However, unlike cobalt and iron, it is normally only stable in aqueous solution in the + 2 oxidation state (Kerfoot, 2002). Selected chemical and physical properties for nickel and nickel compounds, including solubility data, were presented in the previous *IARC Monograph* (IARC, 1990), and have been reported elsewhere (ATSDR, 2005).

1.3 Use of the agents

The chemical properties of nickel (i.e. hardness, high melting point, ductility, malleability, somewhat ferromagnetic, fair conductor of heat and electricity) make it suitable to be combined with other elements to form many alloys (NTP, 2000; Tundermann *et al.*, 2005). It imparts such desirable properties as corrosion resistance, heat resistance, hardness, and strength.

Nickel salts are used in electroplating, ceramics, pigments, and as intermediates (e.g. catalysts, formation of other nickel compounds). Sinter nickel oxide is used in nickel catalysts in the ceramics industry, in the manufacture of alloy steel and stainless steel, in the manufacture of nickel salts for specialty ceramics, and in the manufacture of nickel–cadmium (Ni–Cd) batteries, and nickel–metal-hydride batteries. Nickel sulfide is used as a catalyst in

Table 1.1 Chemical names (CAS names are given in italics), synonyms, and molecular formulae or compositions of nickel, nickel alloys and selected nickel compounds

Chemical name	CAS Reg. No.	Synonyms	Formula
Metallic nickel and nickel alloys			
Nickel	7440-02-0	C.I. 77775; Nickel element	Ni
Ferronickel	11133-76-9	*Iron alloy (base), Fe, Ni*; nickel alloy (nonbase) Fe, Ni	Fe, Ni
Nickel aluminium alloys	61431-86-5 37187-84-1	*Raney nickel*; Raney alloy	NiAl
Nickel oxides and hydroxides			
Nickel hydroxide (amorphous)	12054-48-7 (11113-74-9)	Nickel dihydroxide; nickel (II) hydroxide; nickel (2+) hydroxide; *nickel hydroxide (Ni(OH)2)*; nickelous hydroxide	$Ni(OH)_2$
Nickel monoxide	1313-99-1 11099-02-8 34492-97-2	Black nickel oxide[a]; green nickel oxide; mononickel oxide; nickel monooxide; nickelous oxide; *nickel oxide (NiO)*; nickel (II) oxide; nickel (2+) oxide *Bunsenite (NiO)*	NiO
Nickel trioxide	1314-06-3	Black nickel oxide[d]; dinickel trioxide; nickelic oxide; nickel oxide; nickel (III) oxide; *nickel oxide (Ni_2O_3)*; nickel peroxide; nickel sesquioxide	Ni_2O_3
Nickel sulfides			
Nickel disulfide	12035-51-7 12035-50-6	*Nickel sulfide (NiS_2)* *Vaesite (NiS_2)*	NiS_2
Nickel sulfide (amorphous)	16812-54-7 (11113-75-0) 1314-04-1 (61026-96-8)	Mononickel monosulfide; nickel mono-sulfide; nickel monosulfide (NiS); nickelous sulfide; nickel (II) sulfide; nickel (2+) sulfide; *Nickel sulfide (NiS)* *Millerite (NiS)*	NiS
Nickel subsulfide	12035-72-2 12035-71-1	Nickel sesquisulfide; nickel subsulfide (Ni_3S_2); *nickel sulfide (Ni_3S_2)*; trinickel disulfide *Heazlewoodite (Ni_3S_2)*; Khizlevudite	Ni_3S_2
Pentlandite	53809-86-2 12174-14-0	Pentlandite ($Fe_9Ni_9S_{16}$) Pentlandite	Fe9Ni9S16 $(Fe_{0.4-0.6}Ni_{0.4-0.6})_9S_8$

Table 1.1 (continued)

Chemical name	CAS Reg. No.	Synonyms	Formula
Nickel salts			
Nickel carbonate	3333-67-3	*Carbonic acid, nickel (2+) salt (1:1)*; nickel carbonate (1:1); nickel (II) carbonate; nickel (2+) carbonate; nickel carbonate ($NiCO_3$); nickel (2+) carbonate ($NiCO_3$); nickel monocarbonate; nickelous carbonate	$NiCO_3$
Basic nickel carbonates	12607-70-4	Carbonic acid, nickel salt, basic; nickel carbonate hydroxide ($Ni_3(CO_3)(OH)_4$); *nickel, (carbonato(2-)) tetrahydroxytri-*	$NiCO_3.2Ni(OH)_2$
	12122-15-5	*Nickel bis(carbonato(2-)) hexahydroxypenta-*; nickel hydroxycarbonate	$2NiCO_3.3Ni(OH)_2$
Nickel acetate	373-02-4	*Acetic acid, nickel (2+) salt*; nickel (II) acetate; nickel (2+) acetate; nickel diacetate; nickelous acetate	$Ni(OCOCH_3)_2$
Nickel acetate tetrahydrate	6018-89-9	*Acetic acid, nickel (+2) salt, tetrahydrate*	$Ni(OCOCH_3)_2.4H_2O$
Nickel ammonium sulfates	15-699-18-0	Ammonium nickel sulfate ($(NH_4)_2Ni(SO_4)_2$); nickel ammonium sulfate ($Ni(NH_4)_2(SO_4)_2$); *sulfuric acid, ammonium nickel (2+) salt (2:2:1)*	$Ni(NH_4)_2(SO_4)_2$
Nickel ammonium sulfate hexahydrate	25749-08-0	Ammonium nickel sulfate ($(NH_4)_2Ni_2(SO_4)_3$); *sulfuric acid, ammonium nickel (2+) salt (3:2:2)*	$Ni_2(NH_4)_2(SO_4)_3$
	7785-20-8	Ammonium nickel (2+) sulfate hexahydrate; ammonium nickel sulfate ($(NH_4)_2Ni(SO_4)_2$); diammonium nickel disulfate hexahydrate; diammonium nickel (2+) disulfate hexahydrate; nickel ammonium sulfate ($Ni(NH_4)_2(SO_4)_2$) hexahydrate; nickel diammonium disulfate hexahydrate; *sulfuric acid, ammonium nickel (2+) salt (2:2:1), hexahydrate*	$Ni(NH_4)_2(SO_4)_2.6H_2O$
Nickel chromate	14721-18-7	*Chromium nickel oxide ($NiCrO_4$)*; nickel chromate ($NiCrO_4$); nickel chromium oxide ($NiCrO_4$)	$NiCrO_4$
Nickel chloride	7718-54-9	Nickel (II) chloride; nickel (2+) chloride; *nickel chloride ($NiCl_2$)*; nickel dichloride; nickel dichloride ($NiCl_2$); nickelous cholride	$NiCl_2$
Nickel chloride hexahydrate	7791-20-0	*Nickel chloride (NiCl2) hexahydrate*	$NiCl_2.6H_2O$
Nickel nitrate hexahydrate	13478-00-7	Nickel (2+) bis(nitrate)hexahydrate; nickel dinitrate hexahydrate; nickel (II) nitrate hexahydrate; nickel nitrate (Ni(NO3)2) hexahydrate; nickelous nitrate hexahydrate; *nitric acid, nickel (2+) salt, hexahydrate*	$Ni(NO_3)_2.6H_2O$
Nickel sulfate	7786-81-4	Nickel monosulfate; nickelous sulfate; nickel sulfate (1:1); nickel (II) sulfate; nickel (2+) sulfate; nickel (2+) sulfate (1:1); nickel sulfate ($NiSO_4$); *sulfuric acid, nickel (2+) salt (1:1)*	$NiSO_4$
Nickel sulfate hexahydrate	10101-97-0	*Sulfuric acid, nickel (2+) salt (1:1), hexahydrate*	$NiSO_4.6H_2O$
Nickel sulfate heptahydrate	10101-98-1	*Sulfuric acid, nickel (2+) salt (1:1), heptahydrate*	$NiSO_4.7H_20$

Table 1.1 (continued)

Chemical name	CAS Reg. No.	Synonyms	Formula
Other nickel compounds			
Nickel carbonyl	13463-39-3	*Nickel carbonyl ($Ni(CO)_4$), (T-4)-*; nickel tetracarbonyl; tetracarbonylnickel; tetracarbonylnickel (0)	$Ni(CO)_4$
Nickel antimonide	12035-52-8	*Antimony compound with nickel (1:1)*; nickel antimonide (NiSb); nickel compound with antimony (1:1); nickel monoantimonide	NiSb
	12125-61-0	*Breithauptite (SbNi)*	
Nickel arsenides	27016-75-7	*Nickel arsenide (NiAs)*	NiAs
	1303-13-5	Nickeline; *nickeline (NiAs)*; niccolite	NiAs
	12256-33-6	*Nickel arsenide ($Ni_{11}As_8$)*; nickel arsenide tetragonal	$Ni_{11}As_8$
	12044-65-4	*Maucherite ($Ni_{11}As_8$)*; Placodine; Temiskamite	$Ni_{11}As_8$
	12255-80-0	*Nickel arsenide (Ni_5As_2)*; nickel arsenide hexagonal	Ni_5As_2
Nickel selenide	1314-05-2	Nickel monoselenide; *nickel selenide (NiSe)*	NiSe
	12201-85-3	Maekinenite; *Makinenite (NiSe)*	
Nickel subselenide	12137-13-2	*Nickel selenide (Ni_3Se_2)*	Ni_3Se_2
Nickel sulfarsenide	12255-10-6	*Nickel arsenide sulfide (NiAsS)*	NiAsS
	12255-11-7	*Gersdorffite (NiAsS)*	
Nickel telluride	12142-88-0	Nickel monotelluride; *nickel telluride (NiTe)*	NiTe
	24270-51-7	*Imgreite (NiTe)*	
Nickel titanate	12035-39-1	Nickel titanate(IV); nickel titanate (Ni-TiO3); *nickel titanium oxide (NiTiO3)*; nickel titanium trioxide	$NiTiO_3$
Chrome iron nickel black spinel	71631-15-7	CI: 77 504; *CI Pigment Black 30*; nickel iron chromite black spinel	$(Ni,Fe)(CrFe)_2O_4$ NS
Nickel ferrite brown spinel	68187-10-0	*CI Pigment Brown 34*	$NiFe_2O_4$
Nickelocene	1271-28-9	Bis(η5-2,4-cyclopentadien-1-yl)nickel; di-π-cyclopentadienylnickel; dicyclopentadienyl-nickel; bis(η5-2,4-cyclopentadien-1-yl)-nickel	π-(C5H5)2Ni

[a] In commercial usage, 'black nickel oxide' usually refers to the low-temperature crystalline form of nickel monoxide, but nickel trioxide (Ni_2O_3), an unstable oxide of nickel, may also be called 'black nickel oxide'.

the petrochemical industry or as an intermediate in the metallurgical industry.

According to the US Geological Survey, world use of primary nickel in 2006 was 1.40 million tonnes, a 12% increase over 2005. Stainless steel manufacture accounted for more than 60% of primary nickel consumption in 2006 (USGS, 2008). Of the 231000 tonnes of primary nickel consumed in the USA in 2007, approximately 52% was used in stainless and alloy steel production, 34% in non-ferrous alloys and superalloys, 10% in electroplating, and 4% in other uses. End uses of nickel in the USA in 2007 were as follows: transportation, 30%; chemical industry, 15%; electrical equipment, 10%; construction, 9%; fabricated metal products, 8%; household appliances, 8%; petroleum industry, 7%; machinery, 6%; and others, 7% (Kuck, 2008).

1.3.1 Metallic nickel and nickel alloys

Pure nickel metal is used to prepare nickel alloys (including steels). It is used as such for plating, electroforming, coinage, electrical components, tanks, catalysts, battery plates, sintered components, magnets, and welding rods. Ferronickel is used to prepare steels. Stainless and heat-resistant steels accounted for 93% of its end-use in 1986. Nickel-containing steels with low nickel content (< 5%) are used in construction and tool fabrication. Stainless steels are used in general engineering equipment, chemical equipment, domestic applications, hospital equipment, food processing, architectural panels and fasteners, pollution-control equipment, cryogenic uses, automotive parts, and engine components (IARC, 1990).

Nickel alloys are often divided into categories depending on the primary metal with which they are alloyed (e.g. iron, copper, molybdenum, chromium) and their nickel content. Nickel is alloyed with iron to produce alloy steels (containing 0.3–5% nickel), stainless steels (containing as much as 25–30% nickel, although 8–10% nickel is more typical), and cast irons. Nickel–copper alloys (e.g. Monel alloys) are used for coinage (25% nickel, 75% copper), industrial plumbing (e.g. piping and valves), marine equipment, petrochemical equipment, heat exchangers, condenser tubes, pumps, electrodes for welding, architectural trim, thermocouples, desalination plants, ship propellers, etc. Nickel–chromium alloys (e.g. Nichrome) are used in many applications that require resistance to high temperatures such as heating elements, furnaces, jet engine parts, and reaction vessels. Molybdenum-containing nickel alloys and nickel–iron–chromium alloys (e.g. Inconel) provide strength and corrosion resistance over a wide temperature range, and are used in nuclear and fossil-fuel steam generators, food-processing equipment, and chemical-processing and heat-treating equipment. Hastelloy alloys (which contain nickel, chromium, iron, and molybdenum) provide oxidation and corrosion resistance for use with acids and salts. Nickel-based super-alloys provide high-temperature strength and creep, and stress resistance for use in gas-turbine engines (ATSDR, 2005).

Other groups of nickel alloys are used according to their specific properties for acid-resistant equipment, heating elements for furnaces, low-expansion alloys, cryogenic uses, storage of liquefied gases, high-magnetic-permeability alloys, and surgical implant prostheses.

1.3.2 Nickel oxides and hydroxides

The nickel oxide sinters are used in the manufacture of alloy steels and stainless steels.

Green nickel oxide is a finely divided, relatively pure form of nickel monoxide, produced by firing a mixture of nickel powder and water in air at 1000 °C (IARC, 1990). It is used to manufacture nickel catalysts and specialty ceramics (for porcelain enamelling of steel; in the manufacture of magnetic nickel-zinc ferrites used in electric motors, antennas and television tube yokes; and

as a colourant in glass and ceramic stains used in ceramic tiles, dishes, pottery, and sanitary ware).

Black nickel oxide is a finely divided, pure nickel monoxide, produced by calcination of nickel hydroxycarbonate or nickel nitrate at 600 °C; nickel trioxide (Ni_2O_3), an unstable oxide of nickel, may also be called 'black nickel oxide' (IARC, 1990). Black nickel oxide is used in the manufacture of nickel salts, specialty ceramics, and nickel catalysts (e.g. to enhance the activity of three-way catalysts containing rhodium, platinum, and palladium used in automobile exhaust control).

Nickel hydroxide is used as a catalyst intermediate, and in the manufacture of Ni–Cd batteries (Antonsen & Meshri, 2005).

1.3.3 Nickel sulfides

Nickel sulfide is used as a catalyst in petrochemical hydrogenation when high concentrations of sulfur are present in the distillates. The major use of nickel monosulfide is as an intermediate in the hydrometallurgical processing of silicate-oxide nickel ores (IARC, 1990). Nickel subsulfide is used as an intermediate in the primary nickel industry (ATSDR, 2005).

1.3.4 Nickel salts

Nickel acetate is used in electroplating, as an intermediate (e.g. as catalysts and in the formation of other nickel compounds), as a dye mordant, and as a sealer for anodized aluminium.

Nickel carbonate is used in the manufacture of nickel catalysts, pigments, and other nickel compounds (e.g. nickel oxide, nickel powder); in the preparation of coloured glass; and, as a neutralizing compound in nickel-electroplating solutions.

Nickel ammonium sulfate is used as a dye mordant, in metal-finishing compositions, and as an electrolyte for electroplating.

Nickel chloride is used as an intermediate in the manufacture of nickel catalysts, and to absorb ammonia in industrial gas masks.

Nickel nitrate hexahydrate is used as an intermediate in the manufacture of nickel catalysts and Ni–Cd batteries.

Nickel sulfate hexahydrate is used in nickel electroplating and nickel electrorefining, in 'electroless' nickel plating, and as an intermediate (in the manufacture of other nickel chemicals and catalysts) (Antonsen & Meshri, 2005).

1.3.5 Other nickel compounds

The primary use for nickel carbonyl is as an intermediate (in the production of highly pure nickel), as a catalyst in chemical synthesis, as a reactant in carbonylation reactions, in the vapour-plating of nickel, and in the fabrication of nickel and nickel alloy components and shapes.

Nickelocene is used as a catalyst and complexing agent, and nickel titanate is used as a pigment (Antonsen & Meshri, 2005).

No information was available to the Working Group on the use of nickel selenides or potassium nickelocyanate.

1.4 Environmental occurrence

Nickel and its compounds are naturally present in the earth's crust, and are emitted to the atmosphere via natural sources (such as windblown dust, volcanic eruptions, vegetation forest fires, and meteoric dust) as well as from anthropogenic activities (e.g. mining, smelting, refining, manufacture of stainless steel and other nickel-containing alloys, fossil fuel combustion, and waste incineration). Estimates for the emission of nickel into the atmosphere from natural sources range from 8.5 million kg/year in the 1980s to 30 million kg/year in the early 1990s (ATSDR, 2005). The general population is exposed to low levels of nickel in ambient air, water, food, and through tobacco consumption.

1.4.1 Natural occurrence

Nickel is widely distributed in nature and is found in animals, plants, and soil (EVM, 2002). It is the 24th most abundant element, forming about 0.008% of the earth's crust (0.01% in igneous rocks). The concentration of nickel in soil is approximately 79 ppm, with a range of 4–80 ppm (EVM, 2002; ATSDR, 2005).

1.4.2 Air

Nickel is emitted to the atmosphere from both natural and anthropogenic sources. It has been estimated that approximately 30000 tonnes of nickel may be emitted per year to the atmosphere from natural sources. The anthropogenic emission rate is estimated to be between 1.4–1.8 times higher than the natural emission rate.

The two main natural sources are volcanoes and windblown dust from rocks and soil, estimated to respectively contribute 14000 tonnes/year and 11000 tonnes/year (NTP, 2000; Barbante *et al.*, 2002). Other relatively minor sources include: wild forest fires (2300 tonnes/year), sea salt spray (1300 tonnes/year), continental particulates (510 tonnes/year), marine (120 tonnes/year), and continental volatiles (100 tonnes/year) (Barbante *et al.*, 2002).

Anthropogenic activities release nickel to the atmosphere, mainly in the form of aerosols (ATSDR, 2005). Fossil fuel combustion is reported to be the major contributor of atmospheric nickel in Europe and the world, accounting for 62% of anthropogenic emissions in the 1980s (Barbante *et al.*, 2002; ATSDR, 2005). In 1999, an estimated 570000 tons of nickel were released from the combustion of fossil fuels worldwide (Rydh & Svärd, 2003). Of this, 326 tons were released from electric utilities (Leikauf, 2002). Of the other anthropogenic sources, nickel metal and refining accounted for 17% of total emissions, municipal incineration 12%, steel production 3%, other nickel-containing alloy production 2%, and coal combustion 2% (ATSDR, 2005).

Atmospheric nickel concentrations are higher in rural and urban air (concentration range: 5–35 ng/m^3) than in remote areas (concentration range: 1–3 ng/m^3) (WHO, 2007).

1.4.3 Water

Particulate nickel enters the aquatic environment from a variety of natural and anthropogenic sources. Natural sources include the weathering and dissolution of nickel-containing rocks and soil, disturbed soil, and atmospheric deposition. Anthropogenic sources include: industrial processes (e.g. mining and smelting operations), industrial waste water and effluent (e.g. tailings piles run-off), domestic waste water, and landfill leachate (NTP, 2000; ATSDR, 2005; WHO, 2007). Several factors influence the concentration of nickel in groundwater and surface water including: soil use, pH, and depth of sampling (WHO, 2007). Most nickel compounds are relatively water soluble at low pH (i.e. pH < 6.5). As a result, acid rain tends to increase the mobility of nickel in soil, which, in turn, has a corresponding impact on nickel concentrations in groundwater (NTP, 2000; WHO, 2007).

Based on measurement data from the 1980s, the following average nickel concentrations have been reported for groundwater, seawater and surface water, respectively: < 20 µg/L, 0.1–0.5 µg/L, and 15–20 µg/L (NTP, 2000; ATSDR, 2005). Nickel concentrations as high as 980 µg/L have been measured in groundwater with pH < 6.2 (WHO, 2007). Levels of dissolved nickel ranging from < 1–87 µg/L have been reported in urban storm run-off water samples (ATSDR, 2005).

Nickel concentrations in the range of 6–700 pg/g have been measured in high-altitude snow and ice near the summit of Mont Blanc on the French-Italian border. Seasonal variations were observed, with higher concentrations in the summer layers than in the winter layers.

Nickel levels appeared to be more associated with anthropogenic inputs (e.g. oil combustion from power generation, automobile and truck traffic) than with natural sources, such as rock and soil dust (Barbante *et al.*, 2002).

1.4.4 Soil and sediments

Natural and anthropogenic sources (e.g. mining and smelting, coal fly ash, bottom ash, metal manufacturing waste, commercial waste, atmospheric fall-out and deposition, urban refuse, and sewage sludge) contribute to the levels of nickel found in soil and sediments (NTP, 2000; ATSDR, 2005). Of the nickel emitted to the environment, the largest releases are to the soil. In 2002, estimated releases of nickel and nickel compounds from manufacturing and processing facilities (required to report to the US Toxic Release Inventory Program) were approximately 5530 and 14800 metric tonnes, respectively—accounting for 82% and 87% of estimated total nickel releases to the environment (ATSDR, 2005).

In a study of urban soil quality, a harmonized sampling regime was used to compare concentrations of nickel in six European cities differing markedly in their climate and industrial history. The sites were as far as possible from current point sources of pollution, such as industrial emissions, but all were bordered by major roads, and are thus likely to have been affected by vehicle emissions. To assess the vertical distribution of soil parameters, two depths were sampled at each point: a surface sample at 0–10 cm and a subsurface sample at 10–20 cm. The surface sample mean nickel concentration was in the range of 11–207 mg /kg, and the corresponding mean concentration in the subsurface sample, 10–210 mg/kg (Madrid *et al.*, 2006).

1.5 Human exposure

1.5.1 Exposure of the general population

Ingestion of nickel in food, and to a lesser degree in drinking-water, is the primary route of exposure for the non-smoking general population. Exposure may also occur via inhalation of ambient air and percutaneous absorption (NTP, 2000; ATSDR, 2005; WHO, 2007). The daily intake of nickel from food and beverages varies by foodstuff, by country, by age, and by gender (EVM, 2002; ATSDR, 2005). Data from a study in the USA give estimates of daily dietary intakes in the range of 101–162 µg/day for adults, 136–140 µg/day for males, and 107–109 µg/day for females. Estimates for pregnant and lactating women are higher with average daily intakes of 121 µg/day and 162 µg/day, respectively (ATSDR, 2005). Based on the concordance between different studies of dietary intake, diet is reported to contribute less than 0.2 mg/day (WHO, 2007).

Inhalation of nickel from ambient air is generally a minor route of exposure for the general population. The following daily intakes of nickel have been estimated: less than 0.05 µg/day in the USA; 0.42 µg/day (mean ambient concentration) and 15 µg/day (highest ambient concentration) in the Sudbury basin region in Ontario, Canada; and, 122 µg/day (based on the highest ambient reported nickel concentration) in the Copper Cliff region of Ontario, Canada. These estimates are based on a breathing rate of 20 m^3/day, and nickel concentrations of 2.2 ng/m^3, 21 ng/m^3, 732 ng/m^3, and 6100 ng/m^3, respectively (ATSDR, 2005).

1.5.2 Occupational exposure

Nickel, in the form of various alloys and compounds, has been in widespread commercial use for over 100 years. Several million workers worldwide are exposed to airborne fumes, dusts and mists containing nickel and its compounds. Exposures by inhalation, ingestion or skin

contact occur in nickel-producing industries (e.g. mining, milling, smelting, and refining), as well as in nickel-using industries and operations (e.g. alloy and stainless steel manufacture; electroplating and electrowinning; welding, grinding and cutting). Insoluble nickel is the predominant exposure in nickel-producing industries, whereas soluble nickel is the predominant exposure in the nickel-using industries. Occupational exposure results in elevated levels of nickel in blood, urine and body tissues, with inhalation as the main route of uptake (IARC, 1990; NTP, 2000).

Estimates of the number of workers potentially exposed to nickel and nickel compounds have been developed by the National Institute of Occupational Safety and Health (NIOSH) in the USA and by CAREX (CARcinogen EXposure) in Europe. Based on the National Occupation Exposure Survey (NOES), conducted during 1981–1983, NIOSH estimated that 507681 workers, including 19673 female workers, were potentially exposed to 'Ni, Nickel-MF Unknown' (agent code: 50420) in the workplace (NIOSH, 1990). The following six industries accounted for nearly 60% of exposed workers: 'fabricated metal products' (n = 69984), 'special trade contractors' (n = 55178), 'machinery, except electrical' (n = 55064), 'transportation equipment' (n = 44838), 'primary metal industries' (n = 39467), and 'auto repair, services, and garages' (n = 27686). Based on occupational exposure to known and suspected carcinogens collected during 1990–1993, the CAREX database estimates that 547396 workers were exposed to nickel and nickel compounds in the European Union. Over 83% of these workers were employed in the 'manufacture of fabricated metal products, except machinery and equipment' (n = 195597), 'manufacture of machinery, except electrical' (n = 122985), 'manufacture of transport equipment' (n = 64720), 'non-ferrous base metal industries' (n = 32168), 'iron and steel basic industries' (n = 26504), and 'metal ore mining' (n = 16459). CAREX Canada (2011) estimates that approximately 50000 Canadians are exposed to nickel in the workplace (95% male). Exposed industries include: commercial/industrial machinery and equipment repair/maintenance; architectural, structural metals manufacturing; specialty trade contractors; boiler, tank and shipping container manufacturing; metal ore mining; motor vehicle parts manufacturing; machine shops, turned product, screw, nut and bolt manufacturing; coating, engraving, heat treating and allied activities; iron/steel mills and ferro-alloy manufacturing; non-ferrous metal production and processing.

Historically, metallic nickel exposures tended to be higher in nickel-producing industries than in the nickel-using industries, with estimates of historical mean levels of exposure to inhalable metallic nickel in the range of 0.01–6.0 mg/m^3 and 0.05–0.3 mg /m^3, respectively. However, data from the EU suggest that occasional higher exposures to inhalable metallic nickel may be present in certain industry sectors (Sivulka, 2005).

Data on early occupational exposures to nickel and nickel compounds were summarized in the previous *IARC Monograph* (IARC, 1990). Data from studies and reviews on nickel exposure published since the previous *IARC Monograph* are summarized below for both the nickel-producing and the nickel-using industries.

(a) Studies of nickel-producing industries

Ulrich *et al.* (1991) collected data on several indicators of nickel exposure (stationary and personal air sampling; urinary nickel excretion) among electrolytic nickel production workers in the Czech Republic (formerly, Czechoslovakia). Air samples (n = 52) were collected on membrane filters and analysed by electrothermal atomic absorption spectrometry. Urine samples (n = 140) were collected during the last 4 hours of workers' shifts, and the results were corrected to a standard density of 1.024. In a matched-pair analysis of air and urine samples collected from 18 electrolysis workers, the correlation coefficient

was 0.562; the mean concentration of nickel in urine was 53.3 µg/L (range, 1.73–98.55 µg/L), and the mean concentration in air was 0.187 mg/m^3 (range, 0.002–0.481 mg/m^3).

In a study conducted at a Finnish electrolytic nickel refinery, Kiilunen *et al.* (1997) collected data on nickel concentrations in air, blood, and urine. Stationary samples (n = 141) were collected from 50 locations in the refinery, including those areas where breathing zone samples were taken. Personal (i.e. 8-hour breathing zone) samples were collected over 4 successive work days (n = 157), from the shoulders when no respiratory protection was worn, inside the mask when protective equipment was worn, and inside the mask hanging on the shoulder of the worker when the mask was taken off. Historical occupational hygiene measurements were examined to assess past exposure. Spot urine samples (n = 154) were collected, pre- and post-shift, over 4 successive work days and 1 free day thereafter. Blood samples (n = 64) were collected at the beginning of the study and at the end of the last work shift. A total of 34 workers (of 100) volunteered to participate in the study. Urinary nickel results in the workers were compared with two non-exposed control groups (30 office workers from the refinery and 32 unexposed persons from the Helsinki area). For the stationary samples, nickel concentrations were reported by location as water-soluble nickel, acid-soluble nickel and total nickel (all in µg/m^3). Geometric mean nickel concentrations ranged from: 7.4 µg/m^3 ('other sites') to 451 µg/m^3 (in 'tank house 3′) for water-soluble nickel; 0.5 µg/m^3 ('other sites') to 4.6 µg/m^3 ('solution purification') for acid-soluble nickel; and, 7.6 µg/m^3 ('other sites') to 452 µg/m^3 (in 'tank house 3′). For the breathing zone samples, the range of geometric mean nickel concentrations was 0.2–3.2 µg/m^3 (inside the mask) and 0.6–63.2 µg/m^3 (no mask). Based on a review of historical stationary sampling data, average nickel concentrations varied in the range of 230–800 µg/m^3 over the period 1966–88. Lower concentrations (112–484 µg/m^3) were observed in the early 1990s. Geometric mean after-shift urinary concentrations of nickel were in the range of 0.1–0.8 µmol/L (mask in use) and 0.5–1.7 µmol/L (no mask in use). Urinary nickel concentrations were still elevated after 2- and 4-week vacations. No consistent correlations between airborne nickel concentrations and nickel concentrations in the blood or urine were observed.

Thomassen *et al.* (2004) measured the exposure of 135 copper refinery workers (45 females, 90 males) to copper, nickel and other trace elements at a nickel refinery complex in Monchegorsk, the Russian Federation. Full-shift breathing zone samples were collected for workers in the pyrometallurgical process (n = 138) and in the electrorefining process (n = 123) areas. Workers wore personal samplers for two to four full shifts. IOM samplers were used to assess the inhalable aerosol fraction, and Respicon samplers (3-stage virtual impactors) were used to separate the inhalable fraction into respirable, tracheobronchal, and extrathoracic aerosol fractions. The geometric mean inhalable nickel concentration was in the range of 0.024–0.14 mg/m^3 for samples taken in the pyrometallurgical areas, and 0.018–0.060 mg/m^3 for samples taken in the electrorefining areas (data presented as the sum of the inhalable water-soluble and water-insoluble subfractions). For the inhalable aerosol nickel concentrations observed in the pyrometallurgical process steps, the water-insoluble subfraction contained higher levels than the water-soluble fraction, with geometric means of 59 µg/m^3 and 14 µg/m^3, respectively. In the electrorefining process area, the nickel concentrations in the inhalable subfractions were 14 µg/m^3 (water-soluble) and 10 µg/m^3 (water-insoluble).

Air monitoring was conducted in three areas of a nickel base metal refinery in South Africa (the ball mill area, the copper winning area, and the nickel handling area). Personal breathing zone samples (n = 30) were collected in all areas of the

plant, and were analysed gravimetrically and by inductively coupled plasma mass spectroscopy. The mean time-weighted average concentrations for soluble, insoluble and total nickel dust, respectively, were 44, 51, and 95 μg/m³ in the ball mill area; 395, 400, and 795 μg/m³ in the nickel handling area; and 46, 17, and 63 μg/m³ in the copper winning area (Harmse & Engelbrecht, 2007).

Airborne dust concentrations, nickel concentrations, nickel speciation, and aerosol particle size distributions in two large-scale nickel production facilities were assessed by collecting a total of 46 inhalable samples (30 personal, 16 area), and 28 cascade impactor samples (18 personal, 10 area). Samples were collected using IOM and Marple cascade impactor sampling heads, and analysed gravimetrically. At the first site, inhalable concentrations were in the range of 0.5–9.1 mg/m³ for the personal samples, and 0.2–5.7 mg/m³ for the area samples (median concentrations, 0.7 mg/m³ and 0.4 mg/m³, respectively). Total nickel levels in the personal samples were in the range of 1.8–814.9 μg/m³, and 19.8–2481.6 μg/m³ in the area samples (median concentrations, 24.6 μg/m³ and 92.0 μg/m³, respectively). At the second site, airborne concentrations of inhalable dust were in the range of 1.2–25.2 mg/m³ for the personal samples, and 1.5–14.3 mg/m³ (median concentrations, 3.8 mg/m³ and 2.9 mg/m³, respectively) for the area samples. Total nickel levels were in the range of 36.6–203.4 μg/m³ in the area samples, and 0.2–170.7 μg/m³ in the personal samples (median concentrations, 91.3 and 15.2 μg/m³, respectively) (Creely & Aitken, 2008).

(b) Studies of nickel-using industries

Bavazzano *et al.* (1994) collected air, face, hand, and spot urine samples from 41 male workers in electroplating operations in 25 small factories in the province of Florence, Italy, and compared them to samples collected from non-exposed male subjects (face and hand samples: n = 15 subjects aged 15–60 years old; urine samples: n = 60 subjects aged 22–63 years old). For the airborne nickel measurements, personal exposure were in the range of 0.10–42 μg/m³ (median concentration, 2.3 μg/m³). The median nickel levels in the urine, on the hands, and on the face were, respectively, 4.2 μg/L (range, 0.7–50 μg/L), 39 μg (range, 1.9–547 μg), and 9.0 μg (range, 1.0–86 μg). Median hand, face, and urine nickel levels for the control subjects were, respectively, 0.8 μg (range, 0.0–5.3 μg; n = 15), 0.30 μg (range, 0.0–2.4; n = 15), and 0.7 μg (range, 0.1–2.5 μg; n = 60).

In an occupational hygiene survey of 38 nickel electroplating shops in Finland, exposure to nickel was assessed by questionnaire (n = 163), urine samples (phase 1: n = 145; phase 2: n = 104), bulk samples (n = 30), and air measurements in three representative shops (one clean, one intermediate, one dirty) on 1 day during which urine samples were also being collected. Full-shift breathing zone samples were collected from inside and outside a respirator with filters. In the first phase of the study, average urinary nickel concentration was 0.16 μmol/L (range, 0.0–5.0 μmol/L; n = 145). The range of mean values for different workplaces was 0.01–0.89 μmol/L, and for the median values, 0.02–0.05 μmol/L. For the 97 workers followed in the second phase, urinary nickel concentrations were observed to fluctuate with exposure, with mean nickel concentrations in the range of 0.10–0.11 μmol/L for the morning specimens, and 0.12–0.16 μmol/L for the afternoon specimens. Personal breathing zone nickel concentrations were as follows: 0.5 μg/m³ (hanger worker in the 'clean shop'), 0.7 μg/m³ (worker responsible for maintenance of nickel bath in the 'clean' shop), and in the range of 5.6–78.3 μg/m³ for workers (n = 6) in the 'dirty' shop. In the area samples, nickel concentrations were 26 μg/m³ (near the nickel bath in the 'clean' shop), 11.9–17.8 μg/m³ (in the hanging area of the 'dirty' shop), and 73.3 μg/m³ (beside the nickel bath in the 'dirty' shop) (Kiilunen *et al.*, 1997).

Kiilunen (1997) analysed data from the biomonitoring registry and the occupational hygiene service registry of the Finnish Institute of Occupational Health to examine trends in nickel exposure during 1980–89. A total of 1795 urinary nickel samples (for which it was possible to identify job titles) were examined, along with 260 nickel measurements from the breathing zone of workers for whom job titles were available. Across all job titles, the ranges of mean urinary nickel concentrations, by time period, were as follows: 0.05–0.52 µmol/L for 1980–82, 0.14–0.51 µmol/L for 1983–85, and 0.17–0.87 µmol/L for 1986–89. The two largest occupational groups sampled were platers (n = 503), and welders (n = 463). Mean urinary concentrations for platers, by time period, were 0.35 µmol/L for 1980–82 (range, 0.01–2.95), 0.30 µmol/L for 1983–85 (range, 0.01–2.10), and 0.38 µmol/L for 1986–89 (range, 0.03–2.37). Mean urinary concentrations for welders, by time period, were 0.22 µmol/L for 1980–82 (range, 0.03–1.58), 0.17 µmol/L for 1983–85 (range, 0.03–0.65), and 0.21 µmol/L for 1986–89 (range, 0.01–1.58). Analysis of the breathing zone measurements revealed that 22.1% of all measurements in 1980–82 had exceeded the occupational exposure limit (OEL) of 0.1 mg/m^3. Similar results were seen for the 1983–85 period (24.8%), rising to 30.7% for the 1986–89 period. Job titles with mean values over the OEL in 1983–85 included: grinders (mean, 0.76 mg/m^3, n = 29), one metal worker (0.12 mg/m^3), powder cutters (mean, 0.34 mg/m^3, n = 31), one spray painter (0.20 mg/m^3), and welders (0.17 mg/m^3, n = 72). Mean levels exceeded the OEL in the following four occupational groups during 1986–89: carbon arc chisellers (mean, 0.6 mg/m^3, n = 2), grinders (mean, 0.28 mg/m^3, n = 19), one warm handler (0.18 mg/m^3), and burn cutters (mean, 0.14 mg/m^3, n = 2).

The association between occupational exposure to airborne nickel and nickel absorption was examined by collecting personal breathing zone samples and urine samples from 10 workers at a galvanizing plant in Brazil that uses nickel sulfate. Spot urine samples were collected pre- and post-shift from the nickel-exposed workers over 5 consecutive days, and from 10 non-nickel exposed workers employed at a zinc plant over 3 consecutive days (n = 97 and 55, respectively). Both groups completed a questionnaire on occupational history, health and lifestyle factors; exposed workers also underwent a medical examination. Personal breathing zone samples (first 4 hours of shift) were collected using NIOSH protocols. Geometric mean airborne nickel levels were in the range of 2.8–116.7 µg/m^3, and the urine levels, from samples taken post-shift, were in the range of 4.5–43.2 µg/g creatinine (mean, 14.7 µg/g creatinine) (Oliveira *et al.*, 2000).

Sorahan (2004) examined data on mean (unadjusted) levels of exposure to inhalable nickel at a nickel alloy plant during 1975–2001 in Hereford, the United Kingdom. Data were reported for two time periods: 1975–80 and 1997–2001. Mean nickel levels (unadjusted) for the earlier period were as follows: 0.84 mg/m^3 in the melting, fettling, and pickling areas; 0.53 mg/m^3 in the extrusion and forge, hot strip and rolling, engineering, and melting stores areas; 0.55 mg/m^3 in the machining, hot rolling, Nimonic finishing, and craft apprentice areas; 0.40 mg/m^3 in the roll turning and grinding, cold rolling, cold drawing, wire drawing, and inspection areas; and 0.04 mg/m^3 in the process stock handling, distribution and warehouse areas. The corresponding mean nickel levels (unadjusted) for the latter period were: 0.37 mg/m^3, 0.45 mg/m^3, 0.31 mg/m^3, 0.30 mg/m^3, and 0.29 mg/m^3, respectively.

Eight-hour TWA (8-h TWA) exposures calculated for the period 1997–2001 were 0.33 mg/m^3, 0.31 mg/m^3, 0.16 mg/m^3, 0.16 mg/m^3, and 0.27 mg/m^3, respectively.

Sorahan & Williams (2005) assessed the mortality of workers at a nickel carbonyl refinery in Clydach, the United Kingdom to determine whether occupational exposure to nickel resulted in increased risks of nasal cancer and lung cancer.

Using personal sampling data collected in the 1980s and 1990s, 8-h TWA exposure to total inhalable nickel was calculated, and assigned to six categories of work, based on the predominant species of nickel exposure. The six categories of work were: feed handling and nickel extraction, including kilns (oxide/metallic); pellet and powder production, and shipping (metallic); nickel salts and derivatives, and effluent (metallic/soluble); wet treatment and related processes (metallic/subsulfide/soluble); gas plant (non-nickel); and engineering and site-wide activities that could include any of the preceding work areas. Mean levels of total inhalable nickel dust were in the range of 0.04–0.57 mg/m^3 in the 1980s (n = 1781), and 0.04–0.37 mg/m^3 in the 1990s (n = 1709).

Stridsklev *et al.* (2007) examined the relationship between the concentration of airborne nickel in the occupational environment of grinders (n = 9) grinding stainless steel in Norway and the concentration of nickel in their urine and blood. Grinders either worked in a well ventilated hall of a shipyard or in a small non-ventilated workshop. The sampling protocol was as follows: full-shift personal samples were collected in the breathing zone of grinders over the course of 1 work week; urine samples were collected three times daily for 1 week (first void in the morning, pre- and post-shift); and blood samples were drawn twice daily for 3 days in 1 week (pre- and post-shift). Blood and urine samples were also collected on the Monday morning after a 3-week vacation in the workshop. Grinders also completed a questionnaire to collect information on work history, use of personal protective equipment, and smoking habits. Mean levels of airborne nickel were 18.9 µg/m^3 (range, 1.8–88.6 µg/m^3) in the shipyard, and 249.8 µg/m^3 (range, 79.5–653.6 µg/m^3) in the workshop. Mean blood nickel levels for grinders were 0.87 µg/L (range, < 0.8–2.4 µg/L) in whole blood, and 1.0 µg/L (range, < 0.4–4.1 µg/L) in plasma. Mean urinary nickel levels for grinders were 3.79 µg/g creatinine (range, 0.68–10.6 µg/g creatinine), 3.39 µg/g creatinine (range, 0.25–11.1 µg/g creatinine), and 4.56 µg/g creatinine (range, < 0.53–11.5 µg/g creatinine), from the first void, pre- and post-shift samples, respectively. With the exception of stainless steel welders welding the MIG/MAG-method [Metal Inert Gas-Metal Active Gas], mean urinary nickel levels were higher in grinders than in welders. Mean urinary nickel levels in MIG/MAG welders were 5.9 µg/g creatinine (range, < 0.24–20.5 µg/g creatinine), 3.8 µg/g creatinine (range, 0.33–11.4 µg/g creatinine), and 4.6 µg/g creatinine (range, < 0.25–18.4 µg/g creatinine) from the first void, pre-, and post-shift samples, respectively.

Sivulka & Seilkop (2009) reconstructed historical exposures to nickel oxide and metallic nickel in the US nickel alloy industry from personal and area measurements collected at 45 plants since the 1940s (n = 6986 measurements). Of the measurements included in the database, 96% were personal breathing zone samples, and 4% were stationary area samples. The data provided evidence of a strongly decreasing gradient of airborne total nickel levels from the 1940s to the present.

1.5.3 Dietary exposure

Nickel has been measured in a variety of foodstuffs as "total nickel." Average concentrations are in the range of 0.01–0.1 mg/kg, but can be as high as 8–12 mg/kg in certain foods (EVM, 2002; WHO, 2007). Factors influencing the concentration of nickel in food include the type of food (e.g. grains, vegetables, fruits versus seafood, mother's milk versus cow's milk), growing conditions (i.e. higher concentrations have been observed in food grown in areas of high environmental or soil contamination), and food preparation techniques (e.g. nickel content of cooking utensils, although the evidence for leaching from stainless steel cookware is somewhat mixed) (EVM, 2002; WHO, 2007).

The highest mean concentrations of nickel have been measured in beans, seeds, nuts and grains (e.g. cocoa beans, 9.8 µg/g; soyabeans, 5.2 µg/g; soya products, 5.1 µg/g; walnuts, 3.6 µg/g; peanuts, 2.8 µg/g; oats, 2.3 µg/g; buckwheat, 2.0 µg/g; and oatmeal, 1.8 µg/g). Although nickel concentrations vary by type of foodstuff, average levels are generally within the range of 0.01–0.1 µg/g. Reported ranges for some common food categories are: grains, vegetables and fruits, 0.02–2.7 µg/g; meats, 0.06–0.4 µg/g; seafood, 0.02–20 µg/g; and dairy, < 100 µg/L (EVM, 2002). This variability in nickel content makes it difficult to estimate the average daily dietary intake of nickel (EVM, 2002).

1.5.4 Biomarkers of exposure

Biomarker levels are influenced by the chemical and physical properties of the nickel compound studied, and by the time of sampling. It should be noted that the nickel compounds, the timing of collection of biological samples (normally at the end of a shift), and the analytical methods used differ from study to study, and elevated levels of nickel in biological fluids and tissue samples are mentioned only as indications of uptake of nickel, and may not correlate directly to exposure levels (IARC, 1990).

Atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) are the most common analytical methods used to determine "total nickel" concentrations in biological materials (such as blood, tissues, urine, and faeces). Nickel content can also be measured in other tissues, such as nails and hair, although specific procedures for dissolving the sample must be followed (ATSDR, 2005). The presence of calcium, sodium or potassium interferes with the quantification of nickel in biological samples, and specific techniques (e.g. isotope dilution) must be used to validate nickel measurements (ATSDR, 2005). Serum and urine samples are the most useful biomarkers of recent exposure, reflecting the amount of nickel absorbed in the previous 24–48 hours (NTP, 2000).

Minoia *et al.* (1990) used atomic absorption spectroscopy and neutron activation analysis to determine trace element concentrations of nickel in urine, blood, and serum collected from non-exposed healthy subjects (n = 1237; 635 males, 602 females) from the Lombardy region of northern Italy. The mean nickel level in urine samples (n = 878) was 0.9 µg/L (range, 0.1–3.9 µg/L); in blood samples (n = 36), 2.3 µg/L (range, 0.6–3.8 µg/L); and in serum samples (n = 385), 1.2 µg/L (range, 0.24–3.7 µg/L).

In a Norwegian-Russian population-based health study, human nickel exposure was investigated in the adult population living near a nickel refinery on both sides of the Norwegian-Russian border during 1994–95. Urine samples were collected from inhabitants, aged 18–69 years, of Nikel, Zapolyarny, and Sor-Varanger and also from individuals living more remotely from the Kola Peninsula nickel-producing centres (in the Russian cities of Apatity and Umba, and the Norwegian city of Tromso). A total of 2233 urine specimens were collected and analysed for nickel using electrothermal atomic absorption spectrometry. The highest urinary nickel concentrations were observed in residents of Nikel (median, 3.4 µg/L; mean, 4.9 µg/L; range, 0.3–61.9 µg/L), followed by Umba (median, 2.7 µg/L; mean, 4.0 µg/L; range, 1.0–17.0 µg/L), Zapolyarny (median, 2.0 µg/L; mean, 2.8 µg/L; range, 0.3–24.2 µg/L), Apatity (median, 1.9 µg/L; mean, 2.6 µg/L; range, 0.3–17.0 µg/L), Tromso (median, 1.2 µg/L; mean, 1.4 µg/L; range, 0.3–6.0 µg/L), and Sor-Varanger (median, 0.6 µg/L; mean, 0.9 µg/L; range, 0.3–11.0 g/L). The Russian participants all had a higher urinary nickel average than those from Norway, regardless of geographic location (Smith-Sivertsen *et al.*, 1998).

Ohashi *et al.* (2006) determined reference values for nickel in urine among women of the general population of 11 prefectures in Japan.

A total of approximately 13000 urine samples were collected in 2000–05 from 1000 adult women aged 20–81 years who had no occupational exposure to nickel. Nickel in urine was analysed by graphite furnace atomic absorption spectrometry. The observed geometric mean concentration for nickel was 2.1 μg/L (range, < 0.2–57 μg/L). After correction for creatinine, the geometric mean concentration was reported as 1.8 μg/L (maximum, 144 μg/L).

1.5.5 Other sources of exposure

Nickel, chromium, and cobalt are common causes of allergic contact dermatitis. In the early 1990s it was recommended that household and other consumer products should not contain more than 5 ppm of each of nickel, chromium, or cobalt, and that, for an even greater degree of protection, the ultimate target level should be 1 ppm. In a recent survey, selected consumer products had the following nickel levels (ppm): hand-wash powders, 0.9; heavy duty powders, 0.5; laundry tablets, 0.5; liquid/powder cleaners, 0.4; heavy duty liquids, 0.1; machine/hand-wash liquids, 0.1; hand-wash liquids, 0.1, fine wash liquids, 0.1; and dishwashing liquids, 0.1 (Basketter *et al.*, 2003).

Potential iatrogenic sources of exposure to nickel are dialysis treatment, leaching of nickel from nickel-containing alloys used as prostheses and implants, and contaminated intravenous medications (Sunderman, 1984).

2. Cancer in Humans

The previous *IARC Monograph* was based upon evidence of elevated risk of lung and nasal cancers observed among workers involved in a variety of nickel sulfide ore smelting and nickel refining processes that included high-temperature processing of nickel matte, nickel–copper matte, electrolytic refining, and Mond process refining. The exposures included metallic nickel, nickel oxides, nickel subsulfide, soluble nickel compounds, and nickel carbonyl. These cohort studies were conducted mainly in Canada, Norway, Finland, and in the United Kingdom (IARC, 1990; ICNCM, 1990).

2.1 Cohort studies and nested case–control studies

Since the previous *IARC Monograph*, several studies have extended follow-up to some of the previous cohorts, and have provided additional cohort and nested case–control analyses related mostly to lung cancer risk, and taking into account potential confounding factors as well as mixed exposures to water-soluble and -insoluble nickel compounds. Among the most common occupations with exposure to nickel compounds are stainless steel welders, who are also exposed to chromium (VI) compounds, and other compounds. Although there have been some cohort studies of stainless steel welders, these are not recorded in the present *Monograph* because it is difficult to ascribe any excess risks in these cohorts to nickel compounds specifically. Key results of some of these cohort studies can be found in Table 2.1 of the *Monograph* on chromium (VI) in this volume.

Also, since the previous *IARC Monograph*, experimental evidence has become available that nickel metal dust can become solubilized and bioavailable after inhalation. Consequently, separately classifying nickel and nickel compounds was viewed by the Working Group as not warranted. A similar distinction has not been made for other metals, e.g. beryllium and cadmium, in other *IARC Monographs*. Accordingly, this review did not exclude studies that focused on metallic nickel, unless they, for other reasons, were considered uninformative.

2.1.1 Cancer of the lung

Studies were carried out in nickel smelters and refineries in Canada, Norway (Kristiansand), Finland, and the United Kingdom (Clydach). Because the refining processes differed in the plants, the exposure profiles to various nickel compounds were different across the cohorts. Nonetheless, increased risks for lung cancer were found in cohorts from all of these facilities (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.1.pdf).

High risks for lung cancers were observed among calcining workers in Canada, who were heavily exposed to both sulfidic and oxidic nickel (nickel sulfides and oxides). A high lung cancer rate was also seen among nickel plant cleaners in Clydach who were heavily exposed to these insoluble compounds, with little or no exposure to soluble nickel. The separate effects of oxides and sulfides could not be estimated, however, as high exposure was always either to both, or to oxides together with soluble nickel. Workers in Clydach calcining furnaces and nickel plant cleaners, exposed to high levels of metallic nickel, had high lung cancer risks (see Table 2.1 online). A substantial excess risk for lung cancer among hydrometallurgy workers in Norway was mainly attributed to their exposure to water-soluble nickel. Their estimated exposures to other types of nickel (metallic, sulfidic, and oxidic) were as much as an order of magnitude lower than those in several other areas of the refinery, including some where cancer risks were similar to those observed in hydrometallurgy. High risks for lung cancer were also observed among electrolysis workers at Kristiansand (Norway). These workers were exposed to high estimated levels of soluble nickel and to lower levels of other forms of nickel. Nickel sulfate and nickel chloride (after 1953) were the only or predominant soluble nickel species present in these areas.

An update of the Kristiansand cohort by Andersen *et al.* (1996) demonstrated a dose–response relationship between cumulative exposure to water-soluble nickel compounds and lung cancer ($P < 0.001$) when adjustment was made for age, smoking, and nickel oxide. The risk was increased 3-fold in the highest soluble nickel dose group. A lesser, but positive, effect was seen between cumulative exposure to nickel oxide and risk of lung cancer, also with adjustment for age, cigarette smoking, and exposure to water-soluble nickel (P for trend = 0.05, see Table 2.2).

Subsequent to the Andersen *et al.* (1996) study, an industrial hygiene study re-evaluated exposure among the Norwegian refinery workers based on new information related to nickel species and exposure levels (Grimsrud *et al.*, 2000). Grimsrud *et al.* (2003) updated the lung cancer incidence among the Norwegian nickel refinery workers (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.3.pdf). The strongest gradient for cumulative exposure and lung cancer was found in relation to water-soluble nickel adjusted for cigarette-smoking habits, which was known for 4728 (89%) of the cohort members. Regarding species of water-soluble nickel compounds, the risk from potential exposure to nickel chloride was similar to that for nickel sulfate. The nickel electrolysis process (using nickel sulfate) changed to a nickel-chloride-based process in 1953, and workers hired in 1953 or later had a similar lung cancer risk (standardized incidence ratio [SIR], 4.4; 95%CI: 1.8–9.1) as for those employed in the same area before 1953 when the nickel sulfate was used (SIR, 5.5; 95%CI: 3.0–9.2). Analyses by year of first employment indicated that those initially employed after 1978 continued to demonstrate a significantly elevated risk of lung cancer (SIR, 3.7; 95%CI: 1.2–8.7), suggesting continued exposure to nickel compounds.

Grimsrud *et al.* (2002) conducted a case–control study of lung cancer nested within the

Table 2.2 Relative risks of lung cancer by cumulative exposure to soluble nickel and nickel oxide, considering the two variables simultaneously by multivariate Poisson regression analysis[a]

Variable	Mean exposure (mg/m^3)	Cases	Relative risk	95%CI	Test for linear trend
Soluble nickel					$P < 0.001$
< 1	0.1	86	1.0	Referent	
1–4	2.3	36	1.2	0.8–1.9	
5–14	8.8	23	1.6	1.0–2.8	
≥ 15	28.9	55	3.1	2.1–4.8	
Nickel oxide					$P = 0.05$
< 1	0.4	53	1.0	Referent	
1–4	2.5	49	1.0	0.6–1.5	
5–14	8.3	53	1.6	1.0–2.5	
≥ 15	44.3	45	1.5	1.0–2.2	

[a] Workers with unknown smoking habits were excluded (three cases of lung cancer).
Adjusted for smoking habits and age.
From Andersen *et al.* (1996)

cohort of Norwegian nickel refinery workers (see Table 2.3 online). Exposure groups were determined based on quintiles of the exposure variables in the controls. Analyses by cumulative exposure adjusted for cigarette smoking indicated that odds ratios for lung cancer in the highest cumulative exposure category of water-soluble nickel, sulfidic nickel, metallic nickel, and oxidic nickel were 3.8 (95%CI: 1.6–9.0), 2.8 (95%CI: 1.1–6.7), 2.4 (95%CI: 1.1–5.3), and 2.2 (95%CI: 0.9–5.4), respectively. The trend for cumulative exposure and lung cancer was significant for water-soluble nickel compounds only ($P = 0.002$). There was, however, a high degree of correlation with exposure to nickel and nickel compounds as a whole, making evaluation of the independent effect of individual compounds difficult. Nonetheless, when data were further adjusted for exposure to water-soluble compounds, there were no significant trends in the odds ratios by cumulative exposure to sulfidic, oxidic, or metallic nickel. The odds ratios related to the highest cumulative exposure group for each of these compounds were 1.2 (95%CI: 0.5–3.3), 0.9 (95%CI: 0.4–2.5), and 0.9 (95%CI: 0.3–2.4), respectively (see Table 2.4). In further analyses, with adjustment for cigarette smoking, arsenic, asbestos, sulfuric acid mist, cobalt and occupational carcinogenic exposures outside the refinery, the strong association between lung cancer and water-soluble nickel remained (Grimsrud *et al.*, 2005).

Anttila *et al.* (1998) updated an earlier cohort study of Finnish nickel refinery and copper/nickel smelter workers (Karjalainen *et al.*, 1992). Among refinery workers employed after 1945, who were exposed primarily to nickel sulfate, an excess of lung cancer was observed in the overall cohort (SIR, 2.61; 95%CI: 0.96–5.67), and the lung cancer risk increased with > 20 years of latency (SIR, 3.38; 95%CI: 1.24–7.36, based on six cases). Among smelter workers, lung cancer was also elevated in the overall cohort (SIR, 1.39; 95%CI: 0.78–2.28), and, similarly, a significant increase in lung cancer risk with > 20 years of latency was observed (SIR, 2.00; 95%CI: 1.07–3.42).

There have been three subsequent reports that provide additional information on refinery workers in Wales (the United Kingdom) exposed to nickel carbonyl and other nickel compounds.

Easton *et al.* (1992) carried out an updated analysis of Welsh nickel refinery workers to determine which nickel compounds were responsible for lung cancer among the 2524 workers employed

Table 2.4 Adjusted[a] odds ratios for lung cancer by exposure to sulfidic, oxidic or metallic nickel in a nested case–control study of Norwegian nickel refinery workers observed during 1952–95

Cumulative exposure to nickel[b]	Odds ratio	95% CI
Sulfidic nickel		
Unexposed	1.0	
Low	1.5	0.6–3.9
Low-medium	2.2	0.9–5.5
Medium	1.8	0.7–4.5
Medium-high	1.3	0.5–3.3
High	1.2	0.5–3.3
Likehood ratio test: $P = 0.344$		
Oxidic nickel		
Unexposed	1.0	
Low	1.5	0.6–3.8
Low-medium	1.8	0.7–4.5
Medium	1.4	0.6–3.7
Medium-high	1.5	0.6–3.7
High	0.9	0.4–2.5
Likehood ratio test: $P = 0.406$		
Metallic nickel		
Unexposed	1.0	
Low	1.2	0.5–2.9
Low-medium	1.0	0.5–2.4
Medium	1.0	0.4–2.3
Medium-high	1.0	0.4–2.4
High	0.9	0.3–2.4
Likehood ratio test: $P = 0.972$		

[a] Data were adjusted for smoking habits in five categories (never smoker, former smoker, or current smoker of 1–10, 11–20, or > 20 g/day), and for exposure to water-soluble nickel as a continuous variable with natural log-transformed cumulative exposure values (ln[(cumulative exposure) + 1]).

[b] Categories were generated according to quartiles among exposed control. In each of the three analyses, data were unadjusted for the other two insoluble forms of nickel.

From Grimsrud *et al.* (2002)

for > 5 years before the end of 1969, and followed during 1931–85. The model was based on exposures occurring before 1935, and was adjusted for age at first exposure, duration of exposure, and time since first exposure. For lung cancer, the best fitting model suggested risks for soluble and metallic nickel exposures, and much less (if any) risk for nickel oxide or sulfides. Sorahan & Williams (2005) followed during 1958–2000 a group of 812 workers from the cohort of Welsh nickel refinery workers who were hired between 1953–92, and who had achieved > 5 years of employment. The overall lung cancer SMR was 1.39 (95%CI: 0.92–2.01). For those with > 20 years since the start of employment, lung cancer risk was significantly elevated [SMR, 1.65; 95%CI: 1.07–2.41], indicating an elevated risk of lung cancer among those hired since 1953.

Grimsrud & Peto (2006) combined data from the most recent updates of Welsh nickel refinery workers to assess lung cancer mortality risk by period of initial employment. For those first employed since 1930, an elevated risk was observed for lung cancer (SMR, 1.33; 95%CI: 1.03–1.72). [The Working Group noted that

exposures were dramatically reduced during the 1920s.]

Egedahl *et al.* (2001) updated the mortality data among employees at a hydrometallurgical nickel refinery and fertilizer complex in Fort Saskatchewan, Canada, who had worked for 12 continuous months during 1954–78. Among the 718 men exposed to nickel, the lung cancer SMR was 0.67 (95%CI: 0.24–1.46, based on six deaths). Significant decreases were observed for the 'all causes of death' category (SMR, 0.57; 95%CI: 0.43–0.74), and for the 'all cancer deaths' category (SMR, 0.47; 95%CI: 0.25–0.81). [The Working Group considered the study uninformative for the evaluation of cancer risks due to a substantial healthy worker effect which may have masked excess mortality that was associated with nickel exposure.]

Goldberg *et al.* (1994) conducted a 10-year incidence study and a nested case–control study of a cohort of nickel mining (silicate-oxide ores) and refinery workers in New Caledonia, South Pacific. They observed a significant decrease in the incidence of lung cancer, and this was also observed for other respiratory cancers The results of the case–control study did not show elevated risks for respiratory cancers in relation to low levels of exposure to soluble nickel, nickel sulfide, or metallic nickel. For all three nickel exposures separately, the odds ratios were 0.7.

[The Working Group noted that in most of these studies of lung cancer risk in smelters and refineries, there was exposure to metallic nickel together with exposure to the other forms of nickel (Sivulka, 2005). Only one of these studies involved an attempt to evaluate separately the effect of metallic nickel (Grimsrud *et al.*, 2002).]

Several additional studies of workers with potential exposure to metallic nickel were reviewed by the Working Group. Arena *et al.* (1998) evaluated mortality among workers exposed to "high nickel alloys" in the USA. A recent industrial hygiene analysis indicated that oxidic nickel comprised 85% of the total nickel exposure of these workers, with the rest being mostly metallic nickel (Sivulka & Seilkop, 2009). Compared to US national rates, lung cancer was significantly elevated among white men (SMR, 1.13; 95%CI: 1.05–1.21), among non-white men the SMR was 1.08 (95%CI: 0.85–1.34), and in women 1.33 (95%CI: 0.98–1.78). [The Working Group noted that the lung cancer SMR for the entire cohort combined was 1.13 (95%CI: 1.06–1.21) based on 955 observed deaths.] The authors also calculated SMRs based on local (SMSA) rates for the separate population subgroups. When calculated for the total cohort, the resulting SMR was [1.01; 95%CI: 0.95–1.08]. [The Working Group noted that it is difficult to interpret the use of local rates when the study population was derived from 13 separate areas located throughout the USA, but the use of rates from urban areas could have overestimated the expected number of deaths from lung cancer. The Working Group noted that the overall SMR for lung cancer in this study compared with the national population was statistically significant, and provides some evidence of an association between exposures in these plants and lung cancer. It appears that the primary exposure was to nickel oxide and thus, the study cannot be used to evaluate the specific carcinogenicity of metallic nickel. Analysis of lung cancer by duration of employment did not indicate a dose–response. The Working Group noted that duration of employment is a poor measure of exposure when exposures are known to have declined over time.]

There have also been a series of studies conducted in the French stainless steel industry that involved co-exposure to several known and potential human lung carcinogens, and the most detailed exposure assessment considered nickel and chromium combined (Moulin *et al.* 1990, 1993a, b, 1995, 2000).]

The only cohort of workers exposed to metallic nickel in the absence of other nickel compounds (Oak Ridge cohort) included only 814 workers, and provided little statistical power to evaluate

lung cancer risk (Godbold & Tompkins, 1979; Cragle *et al.*, 1984).

Sorahan (2004) updated the mortality rate among employees manufacturing nickel alloys at the plant in Hereford, the United Kingdom. The study showed a significant decrease for 'all causes of death' (SMR, 0.79), for 'all cancer deaths' (SMR, 0.81), and a non-significant decrease for lung cancer (SMR, 0.87; 95%CI: 0.67–1.11).

Pang *et al.* (1996) evaluated cancer risks among 284 men who were employed for at least 3 months during 1945–75 in a nickel-plating department, and followed through 1993. For lung cancer, the overall SMR was 1.08 (95%CI: 0.54–1.94). For those with > 20 years latency, eight lung cancer deaths were observed versus 6.31 expected [SMR, 1.27; 95%CI: 0.55–2.50].

Several other studies reviewed by Sivulka (2005) had mixed exposure to metallic nickel and other nickel compounds, and provide no evidence on the carcinogenicity of metallic nickel alone. Furthermore, many of the studies cited in the review involved mixed exposures in stainless steel welding and grinding, and manufacturing nickel alloys (Cox *et al.*, 1981; Enterline & Marsh, 1982; references from Tables 5 and 6 of Sivulka, 2005), and therefore were not considered relevant for evaluating the carcinogenicity of nickel and/or nickel compounds.

2.1.2 Cancer of the nasal cavity

Increased risks for nasal cancers were found to be associated with exposures during high-temperature oxidation of nickel matte and nickel-copper matte (roasting, sintering, calcining) in cohort studies in Canada, Norway (Kristiansand), and the United Kingdom (Clydach), with exposures in electrolytic refining in a study in Norway, and with exposures during leaching of nickel-copper oxides in acidic solution (copper plant), and extraction of nickel salts from concentrated solution (hydrometallurgy) in the United Kingdom (see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.5.pdf).

In the Norwegian study, Andersen *et al.* (1996) demonstrated a dose–response relationship between both cumulative exposure to water-soluble nickel and nickel oxide compounds and the risk of nasal cancer. The SIR (compared to the general population) was the highest in the group of workers with the highest cumulative exposure to soluble nickel compounds combined with insoluble nickel compounds (SIR, 81.7; 95%CI: 45–135; based on 15 cases). For workers with the highest cumulative exposure to nickel oxide, the SIR was 36.6 (95%CI: 19.5–62.5; based on 13 cases) (see Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.6.pdf).

An update of nasal cancer in Finnish refinery workers after 20 years since the first exposure to nickel reported an SIR of 67.1 (95%CI: 12–242.0; based on two cases) (Anttila *et al.*, 1998). An additional nasal cancer was observed 2 years after the follow-up period ended, and a fourth potential nasal cancer (classified as a nasopharyngeal cancer, 0.04 expected) was reported during the follow-up period. No nasal cancers were observed among the smelter workers who were exposed primarily to nickel matte, nickel subsulfide, nickel sulfides, and other metals.

Easton *et al.* (1992) attempted to identify the nickel compounds responsible for nasal cancer among 2524 Welsh nickel refinery workers employed for > 5 years before the end of 1969, and followed during 1931–85. As shown in Table 2.7, the risk for nasal cancer was in the range of 73–376 times the expected for those first employed before 1930, based on 67 nasal cancer deaths. A statistical model that fitted to the data on men whose exposures occurred before 1935, and that adjusted for age at first exposure, duration of exposure, and time since first exposure indicated that the soluble nickel effect on nasal cancer risk is the only one significant.

Table 2.7 Observed and expected deaths from nasal sinus cancer (1931–85) by year of first employment

Year first employed	Observed deaths	Expected deaths	SMR	95% CI
< 1920	55	0.15	376	276–477
1920–29	12	0.17	73	36–123
1930–39	1	0.07	14	0.4–80
1940–49	0	0.06	–	–
> 1950	0	0.06	–	–
Total	68	0.45	151	117–192

From Easton *et al.* (1992)

Grimsrud & Peto (2006) combined data from the most recent updates of Welsh nickel refinery workers to assess nasal cancer mortality risk by period of initial employment. For those first employed since 1930, an elevated risk was observed for nasal cancer (SMR, 8.70; 95%CI: 1.05–31.41, based on two observed deaths).

In one study of Swedish Ni–Cd battery workers, three nasal cancer cases versus 0.36 expected were observed (SIR, 8.32; 95%CI: 1.72–24.30) (Järup *et al.*, 1998). Two of these cases occurred among workers exposed to greater than 2 mg/m^3 nickel (SIR, 10.8; 95%CI: 1.31–39.0).

2.1.3 Other cancer sites

Other than for lung cancer and nasal sinus cancer, there is currently no consistency in the epidemiological data to suggest that nickel compounds cause cancer at other sites.

The results of several studies of workers exposed to nickel compounds showed a statistically elevated risk of a site-specific cancer in addition to lung and nasal cancer. A study of sinter plant workers in Canada showed a significantly elevated risk of cancer of the buccal cavity and pharynx (IARC, 1990). In a study in the Norwegian nickel-refining industry, a significant excess of laryngeal cancer was observed among roasting and smelter workers (Magnus *et al.*, 1982).

Stomach cancer was significantly elevated among men employed in a nickel- and chromium-plating factory in the United Kingdom (Burges, 1980). A study of men employed in a nickel-plating department (Pang *et al.*, 1996) showed a significant elevation in stomach cancer. Another study (Anttila *et al.*, 1998) demonstrated a significant excess of stomach cancer among nickel refinery workers.

A study of workers producing alloys with a high nickel content (Arena *et al.*, 1998) demonstrated a significant excess of colon cancer among 'non-white males' (relative risk, 1.92; 95%CI: 1.28–2.76), and a 2-fold risk of kidney cancer among white males employed in 'melting.' However, the excess risk was not associated with length of employment or time since first employment. [The Working Group noted that specific data was not provided in the article.]

A meta-analysis (Ojajärvi *et al.*, 2000) reported a significantly elevated risk for pancreatic cancer that upon further evaluation actually indicated no elevation in risk (Seilkop, 2002).

A population-based case–control study (Horn-Ross *et al.*, 1997) based on self-reported occupational exposure, showed a dose–response relationship between cumulative exposure to nickel compounds/alloys and salivary gland cancer. [The Working Group noted that the author corrected the direction of signs in Table 2 of her report in a subsequent erratum.]

2.2 Synthesis

The Working Group evaluated a large body of evidence and concluded that there is an elevated risk of lung and nasal sinus cancer among nickel refinery workers (IARC, 1990; Andersen *et al.*, 1996; Anttila *et al.*, 1998; Grimsrud & Peto, 2006), and an elevation in lung cancer risk among nickel smelter workers (IARC, 1990; Anttila *et al.*, 1998).

Epidemiological studies have provided evidence for lung cancer related to specific nickel compounds or classes of compounds (based, for example, on water solubility). Evidence for elevated risk of lung cancer in humans was demonstrated specifically for nickel chloride (Grimsrud *et al.*, 2003), nickel sulfate, water-soluble nickel compounds in general (Andersen *et al.*, 1996; Grimsrud *et al.*, 2002, 2003; Grimsrud *et al.*, 2005), insoluble nickel compounds, nickel oxides (Andersen *et al.*, 1996; Anttila *et al.*, 1998; Grimsrud *et al.*, 2003), nickel sulfides (Grimsrud *et al.*, 2002), and mostly insoluble nickel compounds (Andersen *et al.*, 1996).

A study that modelled risks of various nickel compounds and lung cancer risk identified both water-soluble nickel and metallic nickel as contributing to risk (Easton *et al.*, 1992). The largest study addressing worker exposure to metallic nickel (in combination with nickel oxide) showed a small but significant elevation in lung cancer risk (Arena *et al.*, 1998).

Other studies specifically addressing nickel metal exposures were uninformative and did not allow any judgment as to whether such exposures should be considered different with regard to cancer risk. It was not possible to entirely separate various nickel compounds in dose–response analyses for specific nickel compounds. In one analysis, an additional adjustment for water-soluble nickel compounds on risk of lung cancer indicated little association with cumulative exposure to sulfidic, oxidic or metallic nickel. One study of Ni–Cd battery workers exposed to nickel hydroxide and cadmium oxide demonstrated a significant risk of cancer of the nose and nasal sinuses.

On the basis of the Norwegian studies of refinery workers, the evidence is strongest for water-soluble nickel compounds and risk for lung cancer. The confidence of the Working Group in the above findings was reinforced by the availability of information on cigarette smoking for 89% of the Norwegian cohort, and the adjustments made for potential confounding exposures.

3. Cancer in Experimental Animals

Nickel and nickel compounds have been tested for carcinogenicity by intramuscular injection to rats, mice, and rabbits; by repository injections at multiple sites in hamsters, rabbits and mice; by intraperitoneal administration to rats and mice; and by intratracheal instillation, intrapleural, intrarenal, intraocular, inhalation, and subcutaneous exposure to rats.

Particularly relevant studies reviewed in the previous *IARC Monograph* (IARC, 1990) were reconsidered in this evaluation, and summarized in the text.

3.1 Oral administration

3.1.1 Nickel sulfide

In a 2-year multiple dose study, oral nickel sulfate hexahydrate given to male and female rats did not result in carcinogenesis (Heim *et al.*, 2007).

3.1.2 Nickel chloride

Nickel chloride was tested for carcinogenicity by oral administration in female hairless mice (CRL: SK1-hrBR). Mice were exposed to ultraviolet radiation (UVR) alone, nickel chloride alone (given in the drinking-water) and UVR + various concentrations of nickel chloride. Nickel

Table 3.1 Studies of cancer in experimental animals exposed to nickel compounds (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 104 wk Heim *et al.* (2007)	Nickel sulfate hexahydrate 0, 10, 30, 50 mg/kg/d (gavage),[a] 60/group/sex	Keratoacanthoma (tail): M–low dose 15% (numbers not provided)	$P < 0.001$	Age at start, 6 wk 99.9% pure Exposure-related decreased bw in males and females (2 highest dose groups) Exposure-related increased mortality ($P_{trend} < 0.008$) in high dose females but not males
Mouse, CRL: Sk1-hrBR (F) 224 d Uddin *et al.* (2007)	Nickel chloride in drinking-water at 3 wk of age 3 wk later UV treatment (1.0 kJ/m^2) 3 d/wk for 26 wk Groups, number of animals Group 1: Controls, 5 Group 2: UV only, 10 Group 3: 500 ppm, 10 Group 4: UV + 20 ppm, 10 Group 5: UV + 100 ppm, 10 Group 6: UV + 500 ppm, 10 5–10/group	Skin (tumours): Number of tumours/mice at 29 wk Group 1: 0 Group 2: 1.7 ± 0.4 Group 3: 0 Group 4: 2.8 ± 0.9 Group 5: 5.6 ± 0.7 Group 6: 4.2 ± 1.0	Group 5 vs Group2 $P < 0.05$ Group 6 vs Group 2 $P < 0.05$	Age at start, 3 wk Nickel had no effect on growth of the mice Nickel levels in skin increased with dose

[a] vehicle not stated

d, day or days; F, female; M, male; UVR, ultraviolet radiation; vs, versus; wk, week or weeks

chloride alone did not cause skin tumours by itself, but when combined with UVR, it increased the UVR-induced skin tumour incidence (Uddin *et al.*, 2007).

See Table 3.1.

3.2 Inhalation exposure

3.2.1 Nickel sulfate hexahydrate

Nickel sulfate hexahydrate was not shown to be carcinogenic in male or female rats or male or female mice when given by inhalation in a 2-year bioassay study (Dunnick *et al.*, 1995; NTP, 1996a). Analysis of lung burden showed that nickel was cleared from the lungs (Dunnick *et al.*, 1995).

3.2.2 Nickel subsulfide

Nickel subsulfide induced lung tumours in rats exposed by inhalation (Ottolenghi *et al.*, 1975).

Inhalation of nickel subsulfide increased the incidence of aveolar/bronchiolar adenomas and carcinomas in male F344 rats, and increased combined lung tumours in females (Dunnick *et al.*, 1995; NTP, 1996b). Nickel subsulfide also increased the incidence of adrenal pheochromocytomas (benign or malignant) in male and female rats, malignant pheochromocytomas were increased in male rats. Significant dose-related trends were observed for both lung and adrenal tumours in both sexes.

3.2.3 Nickel oxide

The carcinogenicity of nickel oxide was investigated in 2-year inhalation studies in F344 male and female rats, and B6C3F$_1$ male and female mice. Nickel oxide induced tumours of the lung (aveolar bronchiolar adenomas or carcinomas), and adrenal medulla (malignant and benign pheochromocytoma) in both sexes of rats. Nickel oxide also increased the incidence of lung tumours in low-dose females but not in male mice (NTP, 1996c).

3.2.4 Metallic nickel

Inhaled metallic nickel increased the incidence of adrenal pheochromocytomas (benign, malignant, and benign and malignant combined) in male rats and adrenal cortex tumours in female rats (Oller *et al.*, 2008). Dose-related responses were observed for both types of adrenal tumours. No significant increases in lung tumours occurred. Elevated blood levels of nickel indicated that metallic nickel was bioavailable systematically after inhalation (Oller *et al.*, 2008).

3.2.5 Other forms of nickel

Nickel carbonyl induced lung carcinomas after inhalation exposure (Sunderman *et al.*, 1957, 1959).

See Table 3.2.

3.3 Parenteral administration

3.3.1 Nickel subsulfide

(a) Mouse

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies in mice (IARC, 1990).

No increase in lung tumour incidence was observed in male strain A/J mice, 20 or 45 weeks after exposure to various treatment regimens of nickel subsulfide (McNeill *et al.*, 1990). In another study, nickel subsulfide induced injection-site tumours in all three strains of mice, with the order of susceptibility to tumour formation being C3H, B6C3F$_1$, and C57BL6 (Rodriguez *et al.*, 1996). Waalkes *et al.* (2004, 2005) studied the carcinogenic response to nickel subsulfide in MT-transgenic and MT-null mice. Intramuscular administration of nickel subsulfide increased the incidence of injections-site tumours (primarily fibrosarcoma) in MT-transgenic and concordant wild-type mice, and lung tumours in MT-transgenic mice (Waalkes *et al.*, 2004). In MT-null mice and concordant wild-type mice, intramuscular injection of nickel sulfide induced fibrosarcomas as well (Waalkes *et al.*, 2005). MT-expression, either overexpression (MT-transgenic mice) or no expression (MT-null), did not significantly affect the carcinogenic response to nickel.

(b) Rat

Nickel subsulfide induced lung tumours in rats exposed by intratracheal instillation (Pott *et al.*, 1987). Intrarenal injection resulted in dose-related increases in renal cell tumours, and intraocular injection resulted in eye tumours in rats (Jasmin & Riopelle, 1976; Sunderman *et al.*, 1979; Albert *et al.*, 1982; Sunderman, 1983). Implantation of nickel subsulfide pellets into rat heterotropic tracheal transplant caused carcinomas and sarcomas (Yarita & Nettesheim, 1978). Local tumours were also observed in rats tested by intramuscular and intrarenal injection with nickel disulfide or nickel monosulfide (crystalline but not amorphous form), and in rats tested by intramuscular injection with nickel ferrosulfide matte (Sunderman, 1984; Sunderman *et al.*, 1984).

When administered by intrarenal injection to F344 male rats, nickel subsulfide induced renal sarcomas (Kasprzak *et al.*, 1994), which showed metastases to the lung, liver, and spleen. Injection site tumours (rhabdomyosarcoma,

Table 3.2 Studies of cancer in experimental animals exposed to nickel compounds or nickel powder (inhalation exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel sulfate hexahydrate				
Rat, F344 (M, F) 104 wk Dunnick *et al.* (1995), NTP (1996a)	0, 0.125, 0.25, 0.5 mg/m^3 (equivalent to 0, 0.03, 0.06, 0.11 mg nickel/m^3) for 6 h/d, 5 d/wk 63–65/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas or squamous cell carcinomas): M–2[a]/54, 0/53, 1/53, 3/53 F[b]–0/52, 0/53, 0/53, 1/54 Adrenal medulla (pheochromocytomas, benign or malignant[c]): M–16/54, 19/53, 13/53, 12/53 F–2/52, 4/52, 3/52, 3/54		Age at start, 6 wk 22.3% Nickel No treatment-related effects on survival. Mean bw of high-dose females were slightly lower than controls. Nickel lung burden values increased with increasing exposure (at 15 mo, 0.15–1.7 µg Ni/g lung)
Mouse, B6C3F$_1$ (M, F) 104 wk Dunnick *et al.* (1995), NTP (1996a)	0, 0.25, 0.5, 1.0 mg/m^3 (equivalent to 0, 0.06, 0.11, 0.22 mg nickel/ m^3) 6 h/d, 5 d/wk 63–65/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas): M–13/61, 18/61, 7/62, 8/61 F–7/61, 6/60, 10/60, 2/60		Age at start, 6 wk 22.3% Nickel No treatment-related effects on survival. Bw of high-dose males and all exposed female groups were decreased Nickel lung burden (µg Ni/g lung) below limit of detection at 7 and 15 mo interim evaluations

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel subsulfide				
Rat, F344 (M, F) 104 wk Dunnick *et al.* (1995), NTP (1996b)	0, 0.15, 1 mg/m^3 (equivalent to 0, 0.11, 0.73 mg nickel/m^3) 6 h/d, 5 d/wk 63/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas or squamous cell carcinomas): M–0/53, 6/53, 11/53 F–2/53, [a]6/53, 9/53 Adrenal medulla (pheochromocytomas, benign or malignant): M–14/53, 30/53, 42/53 F–3/53, 7/53, 36/53	M: mid dose $P < 0.05$, high dose $P \leq 0.01$, $P_{trend} < 0.01$ F: mid dose $P \leq 0.05$ vs historical control, high dose $P < 0.05$, $P_{trend} < 0.05$ M: mid dose $P < 0.01$, high dose < 0.001, $P_{trend} < 0.001$ F: high dose, $P < 0.001$ $P_{trend} < 0.001$	Age at start, 6 wk 73.3% Nickel No treatment-related effects on survival. Bw in high-dose groups Nickel lung burden increased with increasing exposure but reached steady-state by 15 mo (4–7 µg Ni/g lung). Lung carcinomas also were significantly increased in high-dose males
Mouse, B6C3F$_1$ (M, F) 104 wk Dunnick *et al.* (1995), NTP 1996b	0, 0.6, 1.2 mg/m^3 (equivalent to 0, 0.44, 0.9 mg nickel/m^3) 6 h/d, 5 d/wk 63/group	Lung (aveolar/bronchiolar adenomas or carcinomas): M–13/61, 5/59, 6/58 F–9/58, 2/59, 3/60	$P = 0.038$N[h] mid dose vs control $P = 0.028$N[h] mid dose vs control $P = 0.050$N[h] high dose vs control	Age at start, 6 wk 73.3% Nickel No treatment-related effects on survival. Mean bw lower in exposed groups than control group. Nickel lung burden increased with exposure concentration and with time (at 15 mo, 12–26 µg Ni/g lung)

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 78–80 wk + held 30 wk Ottolenghi *et al.* (1975)	Nickel subsulfide with or without 1 mo pre-exposure to the airborne system (clean air or nickel sulfide dust 0.97 ± 0.18 mg/m^3 for 5 d/wk), followed by injection of hexachlorotetrafluorobutane to half the animals, thereafter the inhalation exposure was continued for all animals 16 exposure groups (8 groups/sex) Pre-exposure Inj. Controls: 29 (M), 28 (F) Inj. NiS: 29 (M), 28 (F) No Inj. Controls: 28 (M), 30 (F) No Inj. NiS: 22 (M), 26 (F) No Pre-exposure Inj. Controls: 32 (M), 32 (F) Inj. NiS: 24 (M), 32 (F) No Inj. Controls: 31 (M), 31 (F) No Inj. NiS: 32 (M), 26 (F)	Lung (adenomas, adenocarcinomas, squamous cell carcinomas, fibrosarcomas): NiS–17 (M), 12 (F) Controls–1 (M), 1 (F) Adrenal gland (hyperplasias and pheochromocytomas): NiS–12% Controls–1.1%	M, F: $P < 0.01$ $P < 0.01$	Pre-exposure: animals assigned airborne system for 1 mo No pre-exposure: animals housed in normal conditions for 1 mo Inj. = intravenous injection with pulmonary infraction agent Treatment-related decreased survival and decreased bw in males and females starting at 26 wk Inflammatory response – pneumonitis, bronchitis and emphysema Hyperplasias and squamous metaplasic changes in bronchial and bronchiolo-alveolar regions Infraction had no effect on carcinogenicity

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel oxide				
Rat, F344 (M, F) 104 wk Dunnick *et al.* (1995), NTP (1996c)	0, 0.62, 1.25, 2.5 mg/m^3 (equivalent to 0, 0.5, 1.0, 2.0 mg nickel/ m^3) 6 h/d, 5 d/wk 65/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas, or squamous cell carcinomas): M–1[a]/54, 1/53, 6/53, 4/52 F–1/53, 0/53[d], 6/53, 5/54	M, F: mid dose & high dose, $P \leq 0.05$ vs high dose	Age at start, 6 wk 76.6% Nickel No treatment-related effects on survival or bw Nickel lung burden increased with exposure and with time (at 15 mo, 262–1116 µg Ni/lung)
		Adrenal medulla (pheochromocytomas, benign or malignant): M–27/54, 24/53, 27/53, 35/54 F[e]–4/51, 7/52, 6/53, 18/54	M: high dose, $P = 0.027$, $P_{trend} = 0.008$ F: high dose, $P = 0.01$, $P_{trend} < 0.001$	If the squamous cell carcinomas (lung tumours) are not included, then the mid dose and high dose are significant vs the current controls Significantly increased incidence of malignant pheochromocytomas in high-dose males
Mouse, B6C3F$_1$ (M, F) 104 wk Dunnick *et al.* (1995), NTP (1996b)	0, 1.25, 2.5, 5.0 mg/m^3 (equivalent to 0, 1.0, 2.0, 3.9 mg nickel/m^3) 6 h/d, 5 d/wk ≈80/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas): M–9/57, 14/67, 15/66, 14/69 F–6/64, 15/66, 12/63, 8/64	F: low dose, $P \leq 0.01$	Age at start, 6 wk; 76.6% Nickel No treatment-related effects on survival or bw Nickel lung burden increased with exposure and with time (at 15 mo, 331–2258 µg Ni/lung)

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel metal powder				
Rat, Wistar Crl:Wi (G1XBRl/Han) (M, F) 12–30 mo Oller *et al.* (2008)	0, 0.1, 0.4, 1 mg/m^3 for 6 h/d, 5 d/wk, exposure time, additional hold time– Group 1: 0, 24 mo, 6 mo Group 2: 0.1, 24 mo, 6 mo Group 3, F: 0.4, 19 mo, 11 mo Group 3, M: 0.4, 24 mo, 6 mo Group 4, F: 1.0, ~14 mo, 0 mo Group 4, M: 1.0, ~12 mo, 0 mo 50/group	Groups 1, 2, 3 Adrenal gland (pheochromocytomas, benign or malignant): M–0/50, 5/50, 21/50 F–0/50, 5/49, 3/53 Adrenal cortex (adenomas or carcinomas): M–1/50, 3/50, 2/50 F–2/50, 2/49, 7/54	M: 0.4 mg/m^3 Significant increase for benign, malignant, benign combined, significant dose-related response[f] F: 0.4 mg/m^3 Significant increase for combined (adenoma and carcinoma) and significant dose-related response[f]	Age at start, 6 wk 99.9% pure Exposure-related mortality was observed in the high-dose group (Group 4 M, F, these animals were removed from the main study), and in Group 3 F (animals from satellite study reassigned to main study). Exposure-related bw effects were observed in Groups 2 (M), 3 (F &M), and 4 (F &M). Exposure-related lung toxicity was observed. Nickel lung burden (µg Ni/lung) increased with exposure and with time (appeared to reach steady-state at 12 mo)[g]. Increases in adrenal tumours were within published (external) historical controls for Wistar rats

[a] Includes 1 squamous cell carcinoma
[b] Only alveolar bronchiolar adenomas observed in female rats; adjusted rate not reported
[c] Adjusted rates not provided
[d] Dunnick reported 1 tumour and NTP technical report reported 0
[e] Only benign tumours observed.
[f] *P*-value not reported calculated by Peto
[g] Data not available for all time points
[h] A negative trend or a lower incidence in an exposure group is indicated by N
bw, body weight; d, day or days; h, hour or hours; F, female; M male; mo, month or months; Ni, nickel; NR, not reported; vs, versus; wk, week or weeks

fibromas, malignant fibrous histiocytomas or leiomyosarcomas) were observed in male or female F344 rats administered nickel subsulfide intramuscularly (Ohmori *et al.*, 1990; Kasprzak & Ward, 1991), and intra-articularly (Ohmori *et al.*, 1990). One study found that in female rats subjected to bone fractures and treated intramuscularly or intra-articularly had a shorter time to sarcoma formation, reduced survival time, and higher metastatic rate than rats treated with nickel alone (Ohmori *et al.*, 1990). Ohmori *et al.* (1999) studied strain susceptibility in male and female Wistar rats, and one strain (CRW) was found to be more sensitive to intramuscular injection of nickel.

(c) Hamster

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies in hamsters (IARC, 1990).

(d) Rabbit

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies rabbits (IARC, 1990).

3.3.2 Nickel oxide and hydroxide

Nickel oxide induced lung tumours in rats by intratracheal instillation (Pott *et al.*, 1987), local sarcomas in mice by intramuscular injection (Gilman, 1962), and rats by intramuscular, intrapleural, and intraperitoneal injection (Gilman, 1962; Sunderman & McCully, 1983; Skaug *et al.*, 1985; Pott *et al.*, 1987). Nickel hydroxide induced local sarcomas in rats when tested by intramuscular injection (Gilman, 1966; Kasprzak *et al.*, 1983).

Sunderman *et al.* (1990) tested the carcinogenicity of five nickel oxides or nickel-copper oxides in male Fisher 344 rats. The three oxides that induced sarcomas at the injection sites had measurable dissolution rates in body fluids, and were strongly positive in an erythrocytosis stimulation assay, demonstrating nickel bioavailability.

3.3.3 Nickel acetate

(a) Mouse

Nickel acetate when administered by intraperitoneal injection induced lung adenocarcinomas and pulmonary adenomas in Strain A mice (Stoner *et al.*, 1976; Poirier *et al.*, 1984).

(b) Rat

Nickel acetate induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats (Pott *et al.*, 1989, 1990).

A single intraperitoneal injection of nickel acetate initiated renal epithelial tumours (including carcinoma) after promotion using sodium barbital in the drinking-water in male rats (Kasprzak *et al.*, 1990).

See Table 3.3.

3.3.4 Metallic nickel

Intratracheal administration of metallic nickel powder caused lung tumours in rats (Pott *et al.*, 1987). Metallic nickel also caused local tumours in rats when administered by injection (intrapleural, subcutaneous, intramuscular, and intraperitoneal) (Hueper, 1952, 1955; Mitchell *et al.*, 1960; Heath & Daniel, 1964; Furst & Schlauder, 1971; Berry *et al.*, 1984; Sunderman, 1984; Judde *et al.*, 1987; Pott *et al.*, 1987, 1990).

3.3.5 Nickel sulfate

Nickel sulfate induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats (Pott *et al.*, 1989, 1990).

Table 3.3 Studies of cancer in experimental animals exposed to nickel compounds (parenteral administration and intratracheal instillation)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel subsulfide				
Mouse, Strain A (M) 45 wk McNeill *et al.* (1990)	i.t. and i.p. 0, 0.53, 0.160 mg/kg bw 3 dosing regimens for 15 wk 1/wk (15 treatments), 1 every 2 wk (8 treatments), 1 every 3 wk (5 treatments); 3 doses per regiment; 30/group 10 mice sacrified after 20 wk	Lung (adenomas at 45 wk[a]): i.t.– Number of treatments: dose 5: 68%, 63%, 58% 8: 64%, 54%, 61% 15: 47%, 47%, 56% i.p.– 5: 68%, 63%, 53% 8: 58%, 53%, 63% 15: 63%, 47%, 50%		Age at start, 8–10 wk Nickel subsulfide –1.8 µm mass medium diameter 73% Nickel and 26.3% sulfur (weight) Urethane (positive control) significantly increased tumour incidence i.p., i.t., after 20 wk, and i.t. after 45 wk, average. number of adenoma/mouse increased i.p. and i.t. at both time points No treatment effects on bw
Mouse, C57BL/6, $B6C3F_1$, CeH/He (M) 78 wk Rodriguez *et al.* (1996)	i.m. (thigh) 0, 0.5, 1.0, 2.5, 5.0, 10 mg/site (single injection) 30/group	Injection site (rhabdomyosarcomas, fibrosarcomas, and other e.g. liposarcomas, haemangiosarcomas): C3He 0/30, 5/30 (16.6%), 10/30 (33.3%), 20/27 (74.1%), 28/29, (96.6%) 14/14 (100%) $B6C3F_1$ 0/30, 2/29 (6.9%), 8/30 (26.7%), 15/30 (50.0%), 16/20 (80%), 5/6 (83.3%) C57BL 0/24, 1/27 (3,7%), 4/28 (14.3%), 6/21 (28.6%), 6/15(40%), 0/2	[$P = 0.052$, 0.5 mg; $P < 0.001$ for other doses][a] [$P < 0.01$, 1.0 mg, $P < 0.001$, 2.5, 5.0, 10 mg][a] [$P < 0.01$, 2.5, 5 mg][a]	Age at start, 6–8 wk; weight, 23–29 g High dose was lethal within 1 wk to over 50% of all 3 strains; susceptibility was C57BL > B6C3F1 > C3H Treatment-related decrease in bw was observed for C3H and $B6C3F_1$ at 2 highest doses. Tumours of the liver, lung adenomas and leukaemias were also observed, but were not increased in exposed groups compared to controls Susceptibility to tumours C3H > $B6C3F_1$ > C57BL

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, MT transgenic and wild-type (M) 104 wk Waalkes *et al.* (2004)	i.m. (both thighs) 0, 0.5, 1 mg/site (single injection) 25/group	Injection site (primarily fibrosarcomas, but also included fibromas and lymphosarcomas): WT–0/24, 5/25 (20%), 10/25 (40%) MT-Tg–0/25, 7/25 (28%), 7/24 (29%) Lung (adenomas or adenocarcinomas): WT–6/24 (25%), 5/25 (20%), 9/25 (36%) MT-Tg–0/25, 3/25 (12%), 4/24 (17%)	WT: $P < 0.05$, mid-and low dose, $P_{trend} < 0.0001$ MT-Tg: $P < 0.05$, mid-and low dose, $P_{trend} = 0.0081$ trend MT-Tg: $P = 0.0502$ high dose $P_{trend} = 0.046$	Age at start, 12 wk 99.9% pure, 30 µm particles Average survival time less in MT-Tg mice than controls. Treatment-related decrease in survival in WT but not MT-Tg mice. No effect on bw No differences in injection-site tumour incidence or latency between MT-Tg and WT mice MT-transgenic controls had significantly lower incidence of lung tumours than WT controls.
Mouse, MT-null (double knockout) and wild-type (M) 104 wk Waalkes *et al.* (2005)	i.m. (both thighs) 0, 0.5, 1 mg/site (single injection), 25/group	Injection site (primarily fibrosarcomas, but also included fibromas): WT–0/24, 8/25(32.0%), 18/25 (72.0%) MT-null–0/24, 11/24 (45.8%), 15/23 (62.5%) Lung (adenomas or adenocarcinomas): WT–7/24 (29.2%), 12/25 (48.0%), 11/25 (44.0%) MT-null–10/24 (41.7%), 13/24 (54.2%), 4/23 (16.7%)	$P < 0.05$ low and high dose $P < 0.05$ low and high dose	Age at start, 12 wk 99.9% pure, < 30 µm particles No difference in survival between control MT-null mice and control WT mice. Nickel treatment reduced survival at later time points corresponding to the appearance of sarcomas. Nickel treatment reduced bw in high-and mid dose MT-null and high-dose WT mice

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, MT-null (double knockout) and wild-type (M) 104 wk Waalkes *et al.* (2005) (contd.)		Lung (adenocarcinomas): WT–1/24 (4.2%), 10/25 (40.0%), 3/25 (12.0%) MT-null–3/24 (12.5%), 3/24 (12.5%), 4/23 (17.4%)	WT: $P < 0.05$ low dose	
		Lung (adenomas): WT–6/24 (25%), 2/25 (8.0%), 8/25 (32.0%)		
		MT-null–7/24 (29.2%), 10/24 (41.7%), 0/23	MT-null: $P < 0.05$ control vs high dose	
Rat, F344/NCr (M) 109 wk Kasprzak *et al.* (1994)	i.r. (2 injections) Ni_3S_2 – 5 mg, MgCarb –6.2 mg, Fe^0–3.4 mg Groups: treatment, number of animals Group 1: Ni_3S_2, 40 Group 2: Ni_3S_2 + MgCarb, 20 Group 3: MgCarb, 20 Group 4: Ni_3S_2+ Fe^0, 20 Group 5: Fe^0, 20 Group 6: vehicle, 20 20–40/group	Kidney (malignant tumours of mesenchymal cell origin) at 104 wk: Group 1: 25/40 (63%) Group 2: 4/20 (20%) Group 3: 0/20 Group 4: 12/20 (60%) Group 5: 0/20 Group 6: 0/20	Group 2 vs Group 1 [$P < 0.01$][a]	Ni_3S_2 < 10µm No effect on bw or survival (from causes other than kidney tumours) MgCarb also delayed onset of tumours (besides decreasing the incidence), and Fe decreased time until first tumour Metastases to lung, liver, spleen and other kidney

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344/NCr (M) 109 wk Kasprzak & Ward (1991)	i.m. and s.c (single injection) Ni_3S_2– 2.5 mg, MB – 0.5 mg, CORT–1.0 mg, IND –1.0 mg. Groups: i.m., s.c., number of animals Group 1: Ni_3S_2, none, 20 Group 2: MB, none, 20 Group 3: Ni_3S_2+ MB, none, 20 Group 4: CORT, none, 20 Group 5: Ni_3S_2+ CORT, none, 20 Group 6: IND, none, 20 Group 7: Ni_3S_2+ IND, none, 20 Group 8: water, none, 20 Group 9: Ni_3S_2, MB, 20 Group 10: Ni_3S_2, IND, 20 20/group	Injection-site tumours (rhabdomosarcomas, fibrosarcomas, histolytic sarcomas): 36 wk; 71 wk Group 1: 10/20 (50%); 17/20 (85%) Group 2: 0/20; 0/20 Group 3:0/20; 1/20 (5%) Group 4: 0/20; 0/20 Group 5: 9/20 (45%); 17/20 (85%) Group 6: 0/20; 0/20 Group 7: 6/20 (30%); 16/20 (80%) Group 8: 0/20; 0/20 Group 9: 18/20 (90%); 20/20 (100%) Group 10: 13/20 (65%); 19/20 (95%)	[Groups 2, 3, 4, 6 or 8 vs Group 1, 36 & 71 wk, $P < 0.01$; Group 9 vs Group 1, 36 wk, $P < 0.05$][a]	Age at start, 8 wk Ni_3S_2 < 10µm No effect on bw Metastases to the lung MB given away from the injection site (s.c.) decreased tumour latency induced by Ni_3S

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (F) 1 yr Ohmori *et. al.* (1990)	Ni_3S_2–10 mg Groups, treatment, number of animals Group 1: fracture bone, 10 mg/fracture, 20 Group 2: 10 mg i.m right thigh, 20 Group 3: 10 mg i.a. right knee joint, 20 Group 4: control (CM), 3 fractured bone, 3 i.m., 2 i.a. 20/group	Injection site (malignant fibrous histiocytomas, rhabdomyosarcomas, fibrosarcomas, leiomyosarcomas): Group 1: 17/20 (85%) Group 2: 20/20 (100%) Group 3: 16/20 (80%) Group 4: 0/7 (0%) Metastasis (lymph node, lung): Group 1: 16/17 (94.1), 9/17 (52.9) Group 2: 5/20 (25.0%), 3/20 (15.0%) Group 3: 3/16 (18.8%), 2/16 (12.5%) Group 4: 0/7, 0/7	$P < 0.05$, Group 1 vs Group 2 or Group 3	Age at start, 10 wk Ni_3S_2 medium particle diameter < 2μm Vehicle, CM Tumour-induction time and survival time shorter in Group 1 than Groups 2 or 3. No osteogenic sarcoma developed in bone-fracture group
Rat, Wistar (M, F) 70 wk Ohmori *et al.* (1999)	Ni_3S_2–10 mg i.m. (single injection) Groups, strain, treatment; number of animals Group 1: SHR–10 mg; 15F, 15M Group 2: CWR–10 mg; 15F, 16M Group 3: SHR–0 mg; 6F, 6M Group 4: CWR–0 mg 7F, 7M 6–15/group	Sarcomas (rhabdomyosarcomas, leiomyosarcomas, fibrosarcomas and malignant fibrous histiocytomas): Groups: F; M; Total Group 1: 2/15 (13.3%); 5/15 (33.3%); 7/30 (23.3%) Group 2: 8/15 (53.3%), 13/16 (81.4%); 21/31 (67.7%) Group 3: 0/6, 0/6 Group 4: 0/7, 0/7	Total: Group 1 vs Group 2, $P < 0.005$	Age, 10 wk Ni_3S_2 medium particle diameter < 2μm Vehicle, CM Tumour incidence, progression (as shown by tumour size and metastasis) was significantly lower in SHR rats (M, F combined) than in CWR rats Metastases observed in the lung and lymph node

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel oxide				
Rat, F344 (M) 104 wk Sunderman *et al.* (1990)	i.m. (hind limb) single injection Group: Ni by wt.; other elements V: vehicle control (glycerol) A: 0.81% Ni (III); none B: 0.05% Ni (III); none F: < 0.03% Ni (III); none H: 21% Cu, 2% Fe, 1.1% Co, 1% S, 0.5% Ni_3S_2 I: 13% Cu, 1.2% Fe, 1.0 Co, 0.3% S, 1.0% Ni_3S_2 (positive control) 20 mg Ni/rat 15/group	Injection site (rhabdomyosarcomas, fibrosarcomas, malignant fibrous histiocytomas, leiomyosarcomas, undifferentiated): V, 0/15; A, 6/15 (40.0%); B, 0/15; F, 0/15; H, 13/15 (86.7%); I, 15/15 (100%) Positive control, Ni_3S_2 15/15(100%) **Metastases** V: 0, A: 3; B: 0; F: 0; H: 4; I: 4 Ni_3S_2: 12 **Other primary tumours** V: 0; A: 0; B: 3; F: 0; H: 0; I: 3 Ni_3S_2: 0	$P < 0.01$ A; $P < 0.001$ H, I, Ni_3S_2	Age at start, ~2 mo 5 NiO compounds – all compounds had 52–79% Nickel (total), and 22–24% O. Nickel could not be determined in Groups H and I because of the presence of sulfur Groups A, H, and I all had measurable dissolution rates in body fluids and were strongly positive in an erythrocytosis-stimulation assay Compounds B and F were insoluble in body fluids, did not stimulate erythrocytosis and had little Ni (III), Cu Fe, Co, or S
Rat, Wistar (F) Life span Pott *et al.* (1987)	(mg x wk) number of animals NiO 50 mg (10 × 5); 34 150 mg (10 × 15); 37 Ni_3S_2 0.94 mg (15 × 0.063); 47 1.88 mg (15 × 0.125); 45 3.75 mg (15 × 0.25); 47 Nickel powder 6 mg (20 × 0.3); 32 9 mg (10 × 0.9); 32 32–47/group	Lung (adenomas, adenocarcinomas, squamous cell carcinomas): % tumours for each dose NiO–27%, 31.6% Ni_3S_2–15%, 28.9% Nickel powder–25.6%, 25% Saline, 0%		Age at start, 11 wk NiO, 99.9% pure

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nickel acetate				
Rat, F344/NCr (M) 101 wk Kasprzak *et al.* (1990)	NiAcet –90 µmol/kg bw single i.p. injection NaBB–50 ppm in drinking-water (2 wk after NiAcet) Groups, treatment, # of animals Group 1: NiAcet, 23 Group 2: NiAcet + NaBB, 24 Group 3: NaBB, 24 Group 4: Saline, 24 24/group	Renal cortical tumours (adenomas & adenocarcinomas): Group 1–1/23 (4.3%) Group 2–16/24 (66.7%) (4 carcinomas) Group 3–6/24 (25%) Group 4–0/24 Renal pelvic tumours (papillomas & carcinomas): Group 1–0/23 Group 2–8/24 (33.3%) Group 3–13/24 (54.2%) (1 carcinoma) Group 4–0/24	$P < 0.008$ vs Group 3	Age at start, 5 wk Initiation/promotion study Decreased survival and bw in rats given nickel acetate followed by NaBB Kidney weight increased in Groups 2 and 3 Renal cortical tumours: metastatic nodules observed in the lung, spleen and liver
Mouse, Strain A (M, F) 30 wk Stoner *et al.* (1976)	i.p. Nickel acetate 3×/wk (24 injections total) 0, 72, 180, 360 mg/kg Saline control 20/group	Lung (adenomas): Average number of tumours/mouse (mean ± SD) Saline: 0.42 ± 0.10 72: 0.67 ± 0.16 180: 0.71 ± 0.19 360: 1.26 ± 0.29	$P < 0.01$ high dose	Age at start, 6–8 wk 99.9% pure Sample of nodules confirmed by histopathology No difference in control M, F, so M, F were combined Positive control urethane Control saline Doses correspond to MTD, ½ MTD, 1/5 MTD
Mouse, Strain A (M, F) 30 wk Poirier *et al.* (1984)	i.p. Nickel acetate 10.7 mg/kg bw (0.04 mmol kg/bw)/injection 3×/wk (24 injections total) 30/group/sex	Lung (adenomas): Average number of tumours/mouse (mean ± SD) Saline: 0.32 ± 0.12 Nickel acetate: 1.50 ± 0.46	$P < 0.05$	Age at start, 6–8 wk Nodules (sample) confirmed by histology Co-exposure to calcium and magnesium decreased multiplicity

[a] Calculated by Fisher Exact Test, Significance not reported by authors

bw, body weight; CM, chloromycetin; CORT, cortisol; CWR, common closed colony rats; F, female; Fe^0, metallic iron; HSR, spontaneously hypertensive rats; i.a., intra-articular; i.f., intra-fat; i.m., intramuscular; IND, indometacin; i.p., intraperitoneal; i.r., intrarenal; i.t., intratracheal instillation; M, male; MB, *Mycobacterium bovis* antigen; MgCarb, magnesium basic carbonate; MT, metallothionien; MTD, maximum tolerated dose; NABB, sodium barbital; Ni, nickel; NiAcet, nickel acetate; Ni_3S, nickel subsulfide; s.c., subcutaneous; SD, standard deviation; Tg, Transgenic; wk, week or weeks; WT, wild type; yr, year or years

Table 3.4 Studies of cancer in experimental animals exposed to nickel acetate (transplacental exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Target organs	Significance	Comments
Rat, F344/NCr (M, F) 85 wk Diwan *et al.* (1992)	*Dams – i.p* NiAcet (90 µmol/kg wt total) Group:/µmol/kg bw; regimen Group 1: 90; once at Day 17 of gestation Group 2: 45; twice at Days 16 & 18 of gestation Group 3: 45; 4 times at Days 12, 14, 16, 18 of gestation Group 4: control (180 NaAcet) once at Day 18 of gestation *Offspring 4 to 85 wk (drinking-water)* ad libitum 1A, 2A, 4A – tap water 1B, 2B, 4B – 0.05% NBB	Renal tumours (cortex adenomas and carcinomas; or pelvis papillomas and carcinomas): 1A: 0/17 (M), 0/16 (F) 2A: 0/15 (M), 0/15 (F) 4A: 0/15 (M), 0/16 (F) 1B: 8/15 (53.3%, M), 0/15 (F) 2B: 7/15 (46.7%, M), 0/15 (F) 4B: 1/15 (6.67%, M), 0/14 (F) Pituitary gland (adenomas or carcinomas): 1A: 9/17 (52.9%, M), 5/16 (31.3%, F), 14/33 (42.3%, M, F) 2A: 6/15 (40.0%, M), 8/16 (50%, F), 14/31 (45.2%, M, F) 4A: 1/15 (6.7%, M), 3/14 (21.4%, F) 1B: 6/15 (40.0%, M), 5/15 (33.3%, F) 2B: 7/15 (46.7%, M), 6/15 (40.0%, F) 4B: 2/15 (13.3%, M), 4/14 (28.6%, F)	M: $P = 0.007$ (1B vs 4B) M: $P = 0.012$ (2B vs 4B) M, F: $P = 0.12$ 1A vs 4A M, F: $P = 0.008$ 2A vs 4A	Dams, age at start 3–4 mo Purity not provided Male (Groups 1 & 2) – significantly decreased bw at 75 wk All offspring in Group 3 died at 72 h. Survival was decreased in Groups 1A, 1B, 2A and 2B compared to controls (4A and 4B) Pituitary tumours: significantly decreased latency for Groups 1A (M, F), 1B (M, F) and 2A (F) compared to the Groups 4A or 4B (corresponding M or F)

h, hour or hours; F, female; i.p., intraperitoneal; M, male; mo, month or months; NaBB, sodium barbital; vs, versus; wk, week or weeks

3.3.6 Nickel chloride

Nickel chloride induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats (Pott *et al.*, 1989, 1990).

3.3.7 Other forms of nickel

Intramuscular administration of nickel sulfarsenide, nickel arsenides, nickel antimonide, nickel telluride, and nickel selenides caused local sarcomas in rats (Sunderman & McCully, 1983). Intramuscular administration of nickelocene caused some local tumours in rats and hamsters (Furst & Schlauder, 1971).

3.4 Transplacental exposure

3.4.1 Nickel acetate

Diwan *et al.* (1992) studied the carcinogenic effects of rats exposed transplacentally to nickel acetate and postnatally to sodium barbital in drinking-water. Pregnant F344 were given nickel acetate by intraperitoneal injection, and their offspring were divided into groups receiving either tap water or sodium barbital in drinking-water. An increased incidence in pituitary tumours was observed in the offspring of both sexes transplacentally exposed to nickel acetate. These tumours were mainly malignant, and are rare tumours. Renal tumours were observed in the male offspring exposed transplacentally to nickel acetate, and receiving sodium barbital postnatally, but not in the male offspring receiving tap water after nickel *in utero*.

See Table 3.4.

3.5 Synthesis

The inhalation of nickel oxide, nickel subsulfide, and nickel carbonyl caused lung tumours in rats. Intratracheal instillation of nickel oxide, nickel subsulfide, and metallic nickel caused lung tumours in rats. Lung tumours were observed by the intraperitoneal injection of nickel acetate in two studies in A/J mice, and by intramuscular injection of nickel subsulfide in mice. The inhalation of nickel oxide, nickel subsulfide, and metallic nickel caused adrenal medulla pheochomocytoma in rats. Transplacental nickel acetate induced malignant pituitary tumours in the offspring in rats. Several nickel compounds (nickel oxides, nickel sulfides, including nickel subsulfide, nickel sulfate, nickel chloride, nickel acetate, nickel sulfarsenide, nickel arsenide, nickel antimonide, nickel telluride, nickel selenide, nickelocene, and metallic nickel) administered by repository injection caused sarcomas in multiple studies. The inhalation of metallic nickel did not cause lung tumours in rats. The inhalation and oral exposure to nickel sulfate did not cause tumours in rats or mice. The inhalation of nickel subsulfite did not cause tumours in mice.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In rodents, nickel salts and nickel sulfides are absorbed through the lungs and excreted mainly in the urine (Benson *et al.*, 1994, 1995a). After inhalation exposure to green nickel oxide, nickel is not distributed in extrapulmonary tissues, and is excreted only in faeces (Benson *et al.*, 1994). In humans, soluble nickel compounds are rapidly absorbed through the lungs, and excreted in the urine. After inhalation exposure to insoluble nickel species, elevated concentrations of nickel are observed in the plasma and urine, but the absorption is slow (Bernacki *et al.*, 1978; Tola *et al.*, 1979).

In rats exposed to nickel sulfate hexahydrate by inhalation for 6 months or 2 years,

no pulmonary accumulation is observed; in a similar exposure scenario with nickel subsulfide, concentrations of nickel are detected in the lungs, with very slight nickel accumulation. Following the exposure of green nickel oxide to rats, the nickel lung clearance half-life is approximately 130 days, and in long-term exposure (NTP, 1996a, b, c; described in Section 3), a remarkable accumulation of nickel is observed (Benson *et al.*, 1995b; Dunnick *et al.*, 1995). The lung clearance half-life of nanoparticulate black nickel oxide in rats is reported as 62 days (Oyabu *et al.*, 2007). The difference in the two clearance rates may be related to the greater water solubility (and the smaller particle size) of the nanoparticulate black nickel oxide. In mice, the observed clearance for nickel sulfate is fast, but for nickel subsulfide intermediate and for green nickel oxide, very slow (Dunnick *et al.*, 1995).

4.1.1 Cellular uptake

Nickel chloride has been shown in different cell lines in culture to be transported to the nucleus (Abbracchio *et al.*, 1982; Edwards *et al.*, 1998; Ke *et al.*, 2006, 2007; Schwerdtle & Hartwig, 2006). Soluble nickel chloride compounds enter cells via the calcium channels and by metal ion transporter 1 (Refsvik & Andreassen, 1995; Funakoshi *et al.*, 1997; Gunshin *et al.*, 1997; Garrick *et al.*, 2006). Crystalline nickel sulfides are phagocytized by a large variety of different cells in culture (Kuehn *et al.*, 1982; Miura *et al.*, 1989; Hildebrand *et al.*, 1990, 1991; IARC, 1990).

Black nickel oxide and nickel chloride are taken up by human lung carcinoma cell lines A549 in culture; the nucleus/cytoplasm ratio is > 0.5 for black nickel oxide, and < 0.18 for nickel chloride (Fletcher *et al.*, 1994; Schwerdtle & Hartwig, 2006).

After phagocytosis of nickel subsulfide, intracellular nickel containing particles rapidly dissolve, and lose sulfur (Arrouijal *et al.*, 1990; Hildebrand *et al.*, 1990, 1991; Shirali *et al.*, 1991).

4.2 Genetic and related effects

The mechanisms of the carcinogenicity of nickel compounds have been reviewed extensively (Hartwig *et al.*, 2002; Zoroddu *et al.*, 2002; Costa *et al.*, 2003, 2005; Harris & Shi, 2003; Kasprzak *et al.*, 2003; Lu *et al.*, 2005; Durham & Snow, 2006; Beyersmann & Hartwig, 2008; Salnikow & Zhitkovich, 2008).

Based on the uptake and distribution in cells described above, the ultimate genotoxic agent is Ni (II). However, direct reaction of Ni (II) with DNA does not seem to be relevant under realistic exposure conditions. Nevertheless, nickel is a redox-active metal that may, in principle, catalyse Fenton-type reactions, and thus generate reactive oxygen species (Nackerdien *et al.*, 1991; Kawanishi *et al.*, 2001). Genotoxic effects have been consistently observed in exposed humans, in experimental animals, and in cell culture systems, and include oxidative DNA damage, chromosomal damage, and weak mutagenicity in mammalian cells. These effects are likely to be due to indirect mechanisms, as described in detail below.

4.2.1 Direct genotoxicity

(a) DNA damage

Water-soluble as well as water-insoluble nickel compounds induce DNA strand breaks and DNA protein crosslinks in different mammalian test systems, including human lymphocytes. Nevertheless, in the case of DNA strand breaks and oxidative DNA lesions, these events mainly occur with conditions that involve comparatively high cytotoxic concentrations (IARC, 1990; Pool-Zobel *et al.*, 1994; Dally & Hartwig, 1997; Cai & Zhuang, 1999; Chen *et al.*, 2003; M'Bemba-Meka *et al.*, 2005; Schwerdtle & Hartwig, 2006; Caicedo *et al.*, 2007). This is also true for the induction of oxidative DNA base modifications in cellular systems. Nevertheless, oxidative DNA damage is also observed in experimental animals, this may

be due to repair inhibition of endogenous oxidative DNA damage.

The intratracheal instillation of several soluble and insoluble nickel compounds to rats significantly increases 8-hydroxydeoxyguanine (8-OH-dG) content in the lungs. Concomitantly, microscopic signs of inflammation in the lungs are also observed. Two distinct mechanisms are proposed: one via an inflammatory reaction and the other through cell-mediated reactive oxygen species formation (Kawanishi *et al.*, 2001; Kawanishi *et al.*, 2002).

(b) Chromosomal alterations

Water-soluble and poorly water-soluble nickel compounds induce sister chromatid exchange and chromosomal aberrations at toxic levels in different mammalian test systems (Conway *et al.*, 1987; Conway & Costa, 1989; IARC, 1990; Howard *et al.*, 1991). Chromosomal aberrations are most pronounced in heterochromatic chromosomal regions (Conway *et al.*, 1987). Water-soluble and poorly water-soluble nickel compounds induce micronuclei at comparatively high concentrations. Because increases in both kinetochore-positive and -negative micronuclei are observed, these effects are likely due to aneugenic as well as clastogenic actions (Arrouijal *et al.*, 1990, 1992; Hong *et al.*, 1997; Seoane & Dulout, 2001). The induction of chromosomal aberrations and micronuclei in rodents treated with different nickel compounds is not consistent across studies (Sobti & Gill, 1989; Arrouijal *et al.*, 1990; Dhir *et al.*, 1991; IARC, 1990; Oller & Erexson, 2007). Enhanced frequencies of chromosomal aberrations were observed in some studies in lymphocytes of nickel-exposed workers (IARC, 1990).

(c) Gene mutations in bacterial and mammalian test systems

Nickel compounds are not mutagenic in bacterial test systems, and are only weakly mutagenic in cultured mammalian cells. Even though, mutagenic responses for both water-soluble and water-insoluble nickel compounds have been reported in transgenic G12 cells, this effect was later shown to result from epigenetic gene-silencing (Lee *et al.*, 1995). Nevertheless, the prolonged culture of V79 cells after treatment with nickel sulfate results in the appearance of genetically unstable clones with high mutation rates together with chromosomal instability (Little *et al.*, 1988; Ohshima, 2003).

(d) Cell transformation

Water-soluble and poorly water-soluble nickel compounds induced anchorage-independent growth in different cell systems (IARC, 1990), including the mouse-embryo fibroblast cell-line PW and the human osteoblast cell line HOS-TE85 (Zhang *et al.*, 2003). Nickel compounds were shown to cause morphological transformation in different cell types (Conway & Costa, 1989; Miura *et al.*, 1989; Patierno *et al.*, 1993; Lin & Costa, 1994).

4.2.2 Indirect effects related to genotoxicity

As stated above, the direct interaction of nickel compounds with DNA appears to be of minor importance for inducing a carcinogenic response. However, several indirect mechanisms have been identified, which are discussed below.

(a) Oxidative stress

Treatment with soluble and insoluble nickel causes increases in reactive oxygen species in many cell types (Huang *et al.*, 1993; Salnikow *et al.*, 2000; Chen *et al.*, 2003).

Increased DNA stand breaks, DNA–protein crosslinks and sister chromatid exchange are found in cells treated with soluble and insoluble nickel compounds, and these are shown to result from the increase in reactive oxygen species (Chakrabarti *et al.*, 2001; Błasiak *et al.*, 2002; Woźniak & Błasiak, 2002; M'Bemba-Meka *et al.*, 2005, 2007).

Intraperitoneal injection of nickel acetate in rat did not cause any DNA damage in liver and kidney at 12 hours. However, oxidative DNA damage increased after 24 hours, and persisted in the kidney for 14 days (Kasprzak *et al.*, 1997).

(b) Inhibition of DNA repair

The treatment of cells with soluble Ni (II) increases the DNA damage and the mutagenicity of various agents (Hartwig & Beyersmann, 1989; Snyder *et al.*, 1989; Lee-Chen *et al.*, 1993).

Soluble Ni (II) inhibits nucleotide-excision repair after UV irradiation, and the effect seems to be on the incision, the polymerization, and ligation steps in this pathway (Hartwig *et al.*, 1994; Hartmann & Hartwig, 1998; Woźniak & Błasiak, 2004). One of the proteins in nucleotide-excision repair, the XPA protein, may be a target of Ni (II) (Asmuss *et al.*, 2000a, b).

Soluble nickel chloride also inhibits base-excision repair. The base-excision repair enzyme, 3-methyladenine-DNA glycosylase II, is inhibited specifically (Dally & Hartwig, 1997; Woźniak & Błasiak, 2004; Wang *et al.*, 2006).

There is some evidence that the enzyme O^6-methylguanine-DNA methyltransferase (MGMT) is inhibited by nickel chloride (Iwitzki *et al.*, 1998).

(c) Epigenetic mechanisms

Both water-soluble and water-insoluble nickel compounds are able to cause gene silencing (Costa *et al.*, 2005). This effect was first found when "mutations" in the transgenic *gpt* gene in G12 cells were found to be epigenetically silenced rather than mutated (Lee *et al.*, 1995). Genes that are located near heterochromatin are subject to such inactivation by nickel. The *gpt* gene was silenced by DNA methylation. Additional studies show that cells treated with nickel have decreased histone acetylation, and altered histone methylation patterns (Golebiowski & Kasprzak, 2005; Chen *et al.*, 2006). Nickel also causes ubiquination and phosphorylation of histones (Karaczyn *et al.*, 2006; Ke *et al.*, 2008a, b). Permanent changes in gene expression are important in any mechanism of carcinogenesis.

4.3 Synthesis

The ultimate carcinogenic species in nickel carcinogenesis is the nickel ion Ni (II). Both water-soluble and poorly water-soluble nickel species are taken up by cells, the former by ion channels and transporters, the latter by phagocytosis. In the case of particulate compounds, nickel ions are gradually released after phagocytosis. Both water-soluble and -insoluble nickel compounds result in an increase in nickel ions in the cytoplasm and the nucleus. Nickel compounds are not mutagenic in bacteria, and only weakly mutagenic in mammalian cells under standard test procedures, but can induce DNA damage, chromosomal aberrations, and micronuclei *in vitro* and *in vivo*. However, delayed mutagenecity and chromosomal instability are observed a long time after treatment of cells with nickel. Nickel compounds act as co-mutagens with a variety of DNA-damaging agents. Thus, disturbances of DNA repair appear to be important. A further important mechanism is the occurrence of epigenetic changes, mediated by altered DNA methylation patterns, and histone modification. Inflammation may also contribute to nickel-induced carcinogenesis.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of mixtures that include nickel compounds and nickel metal. These agents cause cancers of the lung and of the nasal cavity and paranasal sinuses.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nickel monoxides, nickel hydroxides, nickel sulfides (including

nickel subsulfide), nickel acetate, and nickel metal.

There is *limited evidence* in experimental animals for the carcinogenicity of nickelocene, nickel carbonyl, nickel sulfate, nickel chloride, nickel arsenides, nickel antimonide, nickel selenides, nickel sulfarsenide, and nickel telluride.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nickel titanate, nickel trioxide, and amorphous nickel sulfide.

In view of the overall findings in animals, there is *sufficient evidence* in experimental animals for the carcinogenicity of nickel compounds and nickel metal.

Nickel compounds are *carcinogenic to humans (Group 1).*

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ASBESTOS (CHRYSOTILE, AMOSITE, CROCIDOLITE, TREMOLITE, ACTINOLITE, AND ANTHOPHYLLITE)

Asbestos was considered by previous IARC Working Groups in 1972, 1976, and 1987 (IARC, 1973, 1977, 1987a). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Asbestos is the generic commercial designation for a group of naturally occurring mineral silicate fibres of the serpentine and amphibole series. These include the serpentine mineral chrysotile (also known as 'white asbestos'), and the five amphibole minerals – actinolite, amosite (also known as 'brown asbestos'), anthophyllite, crocidolite (also known as 'blue asbestos'), and tremolite (IARC, 1973; USGS, 2001). The conclusions reached in this *Monograph* about asbestos and its carcinogenic risks apply to these six types of fibres wherever they are found, and that includes talc containing asbestiform fibres. Erionite (fibrous aluminosilicate) is evaluated in a separate *Monograph* in this volume.

Common names, Chemical Abstracts Service (CAS) Registry numbers and idealized chemical formulae for the six fibrous silicates designated as 'asbestos' are presented in Table 1.1. Specific chemical and physical properties are also presented.

1.2 Chemical and physical properties of the agent

The silicate tetrahedron (SiO_4) is the basic chemical unit of all silicate minerals. The number of tetrahedra in the crystal structure and how they are arranged determine how a silicate mineral is classified.

Serpentine silicates are classified as 'sheet silicates' because the tetrahedra are arranged to form sheets. Amphibole silicates are classified as 'chain silicates' because the tetrahedra are arranged to form a double chain of two rows aligned side by side. Magnesium is coordinated with the oxygen atom in serpentine silicates. In amphibole silicates, cationic elements such as aluminium, calcium, iron, magnesium, potassium, and sodium are attached to the tetrahedra. Amphiboles are distinguished from one another by their chemical composition. The chemical formulas of asbestos minerals are idealized. In

Table 1.1 Common names, CAS numbers, synonyms, non-asbestos mineral analogues, idealized chemical formulae, selected physical and chemical properties of asbestos minerals

Common Name	CAS No.	Synonyms	Non-Asbestos Mineral Analogue	Idealized Chemical Formula	Colour	Decomposition Temperature (°C)	Other Properties
Asbestos	1332-21-4*	Unspecified		Unspecified			
Serpentine group of minerals							
Chrysotile	12001-29-5*	Serpentine asbestos; white asbestos	Lizardite, antigorite	$[Mg_3Si_2O_5(OH)_4]_n$	White, grey, green, yellowish	600–850	Curled sheet silicate, hollow central core; fibre bundle lengths = several mm to more than 10 cm; fibres more flexible than amphiboles; net positive surface charge; forms a stable suspension in water; fibres degrade in dilute acids
Amphibole group of minerals							
Crocidolite	12001-28-4*	Blue asbestos	Riebeckite	$[NaFe^{2+}_3Fe^{3+}_2Si_8O_{22}(OH)_2]_n$	Lavender, blue green	400–900	Double chain silicate; shorter, thinner fibres than other amphiboles, but not as thin as chrysotile; fibre flexibility: fair to good; spinnability: fair; resistance to acids: good; less heat resistance than other asbestos fibres; usually contains organic impurities, including low levels of PAHs; negative surface charge in water
Amosite	12172-73-5*	Brown asbestos	Grunerite	$[(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2]_n$	Brown, grey, greenish	600–900	Double chain silicate; long, straight, coarse fibres; fibre flexibility: somewhat; resistance to acids: somewhat; occurs with more iron than magnesium; negative surface charge in water
Anthophyllite	17068-78-9*	Ferroanthophyllite; azbolen asbestos	Anthophyllite	$[(Mg, Fe^{2+})_7Si_8O_{22}(OH)_2]_n$	Grey, white, brown-grey, green	NR	Double chain silicate; short, very brittle fibres; resistance to acids: very; relatively rare; occasionally occurs as contaminant in talc deposits; negative surface charge in water
Actinolite	12172-67-7*	Unspecified	Actinolite	$[Ca_2(Mg, Fe^{2+})_5Si_8O_{22}(OH)_2]_n$	Green	NR	Double chain silicate; brittle fibres; resistance to acids: none; occurs in asbestiform and non-asbestiform habit; iron-substituted derivative of tremolite; common contaminant in amosite deposits; negative surface charge in water
Tremolite	14567-73-8*	Silicic acid; calcium magnesium salt (8:4)	Tremolite	$[Ca_2Mg_5Si_8O_{22}(OH)_2]_n$	White to pale green	950–1040	Double chain silicate; brittle fibres; acid resistant; occurs in asbestiform and non-asbestiform habit; common contaminant in chrystotile and talc deposits; negative surface charge in water

* identified as asbestos by CAS Registry
NR, not reported
From ATSDR (2001), USGS (2001), HSE (2005), NTP (2005)

natural samples, the composition varies with respect to major and trace elements (USGS, 2001; HSE, 2005). More detailed information on the chemical and physical characteristics of asbestos – including atomic structure, crystal polytypes, fibre structure, chemistry and impurities – can be found in the previous *IARC Monograph* (IARC, 1973).

The structure of silicate minerals may be fibrous or non-fibrous. The terms 'asbestos' or 'asbestiform minerals' refer only to those silicate minerals that occur in polyfilamentous bundles, and that are composed of extremely flexible fibres with a relatively small diameter and a large length. These fibre bundles have splaying ends, and the fibres are easily separated from one another (USGS, 2001; HSE, 2005). Asbestos minerals with crystals that grow in two or three dimensions and that cleave into fragments, rather than breaking into fibrils, are classified as silicate minerals with a 'non-asbestiform' habit. These minerals may have the same chemical formula as the 'asbestiform' variety. (NIOSH, 2008).

Chrysotile, lizardite, and antigorite are the three principal serpentine silicate minerals. Of these, only chrysotile occurs in the asbestiform habit. Of the amphibole silicate minerals, amosite and crocidolite occur only in the asbestiform habit, while tremolite, actinolite and anthophyllite occur in both asbestiform and non-asbestiform habits (USGS, 2001; HSE, 2005; NTP, 2005).

Historically, there has been a lack of consistency in asbestos nomenclature. This frequently contributed to uncertainty in the specific identification of asbestos minerals reported in the literature. The International Mineralogical Association (IMA) unified the current mineralogical nomenclature under a single system in 1978. This system was subsequently modified in 1997 (NIOSH, 2008).

Asbestos fibres tend to possess good strength properties (e.g. high tensile strength, wear and friction characteristics); flexibility (e.g. the ability to be woven); excellent thermal properties (e.g. heat stability; thermal, electrical and acoustic insulation); adsorption capacity; and, resistance to chemical, thermal and biological degradation (USGS, 2001; NTP, 2005).

1.3 Use of the agent

Asbestos has been used intermittently in small amounts for thousands of years. Modern industrial use dates from about 1880, when the Quebec chrysotile fields began to be exploited. During the next 50 years gradual increases in production and use were reported with a cumulative total of somewhat less than 5000 million kg mined by 1930 (IARC, 1973).

As described above, asbestos has several chemical and physical properties that make it desirable for a wide range of industrial applications. By the time industrial and commercial use of asbestos peaked, more than 3000 applications or types of products were listed (NTP, 2005). Production and consumption of asbestos has declined in recent years due to the introduction of strict regulations governing exposure and/or outright bans on exposure.

Asbestos is used as a loose fibrous mixture, bonded with other materials (e.g. Portland cement, plastics and resins), or woven as a textile (ATSDR, 2001). The range of applications in which asbestos has been used includes: roofing, thermal and electrical insulation, cement pipe and sheets, flooring, gaskets, friction materials (e.g. brake pads and shoes), coating and compounds, plastics, textiles, paper, mastics, thread, fibre jointing, and millboard (USGS, 2001; NTP, 2005; Virta, 2006). Certain fibre characteristics, such as length and strength, are used to determine the most appropriate application. For example, longer fibres tend to be used in the production of textiles, electrical insulation, and filters; medium-length fibres are used in the production of asbestos cement pipes and sheets, friction materials (e.g. clutch facings, brake linings), gaskets, and pipe coverings; and,

short fibres are used to reinforce plastics, floor tiles, coatings and compounds, and roofing felts (NTP, 2005).

Since peaking in the 1970s, there has been a general decline in world production and consumption of asbestos. Peak world production was estimated to be 5.09 million metric tons in 1975, with approximately 25 countries producing asbestos and 85 countries manufacturing asbestos products (USGS, 2001; Nishikawa *et al.*, 2008). Worldwide 'apparent consumption' of asbestos (calculated as production plus imports minus exports) peaked at 4.73 million metric tons in 1980. Asbestos cement products are estimated to have accounted for 66% of world consumption in that year (Virta, 2006). In the USA, consumption of asbestos peaked in 1973 at 719000 metric tons (USGS, 2001).

Historical trends worldwide in per capita asbestos use are presented in Table 1.2, and peak use of asbestos was higher and occurred earlier in the countries of Northern and western Europe, Oceania, and the Americas (excluding South America). Very high asbestos use was recorded in Australia (5.1 kg per capita/year in the 1970s), Canada (4.4 kg per capita/year in the 1970s), and several countries of Northern and western Europe (Denmark: 4.8 kg per capita/year in the 1960s; Germany: 4.4 kg per capita/year in the 1970s; and Luxembourg: 5.5 kg per capita/year in the 1960s) (Nishikawa *et al.*, 2008).

Current use of asbestos varies widely. While some countries have imposed strict regulations to limit exposure and others have adopted bans, some have intervened less, and continue to use varying quantities of asbestos (Table 1.2). According to recent estimates by the US Geological Survey, world production of asbestos in 2007 was 2.20 million metric tonnes, slightly increased from 2.18 million metric ton in 2006. Six countries accounted for 96% of world production in 2006: the Russian Federation (925000 metric tons), the People's Republic of China (360000 metric tons), Kazakhstan (300000 metric tons), Brazil (227304 metric tons), Canada (185000 metric tons), and Zimbabwe (100000 metric tons) (Virta, 2008). During 2000–03, asbestos consumption increased in China, India, Kazakhstan, and the Ukraine (Virta, 2006). 'Apparent' world consumption of asbestos was 2.11 million metric tons in 2003, with the Russian Federation, several former Russian states and countries in Asia being the predominant users (Virta, 2006). Consumption of asbestos in the USA (predominantly chrysotile) was 2230 metric tons in 2006, declining to 1730 metric tons in 2007 (Virta, 2008). Roofing products (includes coatings and compounds) accounted for over 80% of asbestos consumption in the USA (Virta, 2008; Virta, 2009). Asbestos products were banned in all the countries of the European Union, including Member States of eastern Europe, effective January 1, 2005 (EU, 1999).

1.4 Environmental occurrence

1.4.1 Natural occurrence

Asbestos minerals are widespread in the environment, and are found in many areas where the original rock mass has undergone metamorphism (ATSDR, 2001; USGS, 2001). Examples include large chrysotile deposits in the Ural Mountains in the Russian Federation, in the Appalachian Mountains in the USA, and in Canada (Virta, 2006). They may occur in large natural deposits or as contaminants in other minerals (e.g. tremolite asbestos may occur in deposits of chrysotile, vermiculite, and talc). The most commonly occurring form of asbestos is chrysotile, and its fibres are found as veins in serpentine rock formations. Asbestiform amphiboles occur in relatively low quantities throughout the earth's crust and their chemical composition reflects the environment in which they form (Virta, 2002). Although most commercial deposits typically contain 5–6% of asbestos, a few deposits, such

Table 1.2 Historical trend in asbestos use per capita and status of national ban

	Use of asbestos[a] (kg per capita/year)						
Country	**1950s**	**1960s**	**1970s**	**1980s**	**1990s**	**2000s**	**National ban[b]**
Asia							
Israel	3.13	2.87	1.23	0.78	0.44	0.02	No ban
Japan	0.56	2.02	2.92	2.66	1.81	0.46	2004
Others[c] (n = 39)	0.06	0.15	0.25	0.27	0.30	0.31	3/39
Eastern Europe and Southern Europe							
Croatia	0.39	1.13	2.56	2.36	0.95	0.65	No ban
Czech Republic	1.62	2.36	2.91	2.73	1.30	0.14	2005
Hungary	0.76	1.23	2.87	3.29	1.50	0.16	2005
Poland	0.36	1.24	2.36	2.09	1.05	0.01	1997
Romania	ND	ND	1.08	0.19	0.52	0.55	2007
Spain	0.32	1.37	2.23	1.26	0.80	0.18	2002
Others[c] (n = 15)	0.79	1.57	2.35	2.05	2.35	1.72	5/15
Northern Europe and Western Europe							
Austria	1.16	3.19	3.92	2.08	0.36	0.00	1990
Denmark	3.07	4.80	4.42	1.62	0.09	NA	1986
Finland	2.16	2.26	1.89	0.78	ND	0	1992
France	1.38	2.41	2.64	1.53	0.73	0.00	1996
Germany	1.84	2.60	4.44	2.43	0.10	0.00	1993
Iceland	0.21	2.62	1.70	0.02	0	0.00	1983
Lithuania	ND	ND	ND	ND	0.54	0.06	2005
Luxembourg	4.02	5.54	5.30	3.23	1.61	0.00	2002
Netherlands	1.29	1.70	1.82	0.72	0.21	0.00	1994
Norway	1.38	2.00	1.16	0.03	0	0.00	1984
Sweden	1.85	2.30	1.44	0.11	0.04	NA	1986
United Kingdom	2.62	2.90	2.27	0.87	0.18	0.00	1999
Others[c] (n = 5)	3.05	4.32	4.05	2.40	0.93	0.05	5/5

as the Coalinga chrysotile deposits in California, USA, are reported to contain 50% or more (USGS, 2001; Virta, 2006).

1.4.2 Air

Asbestos is not volatile; however, fibres can be emitted to the atmosphere from both natural and anthropogenic sources. The weathering of asbestos-bearing rocks is the primary natural source of atmospheric asbestos. No estimates of the amounts of asbestos released to the air from natural sources are available (ATSDR, 2001). Anthropogenic activities are the predominant source of atmospheric asbestos fibres. Major anthropogenic sources include: open-pit mining operations (particularly drilling and blasting); crushing, screening, and milling of the ore; manufacturing asbestos products; use of asbestos-containing materials (such as clutches and brakes on cars and trucks); transport and disposal of wastes containing asbestos; and, demolition of buildings constructed with asbestos-containing products, such as insulation, fireproofing, ceiling and floor tiles, roof shingles, drywall, and cement (ATSDR, 2001; NTP, 2005). Concentrations of asbestos vary on a site-by-site basis and, as a result, environmental emissions are not easily estimated (ATSDR, 2001).

Table 1.2 (continued)

	Use of asbestos[a] (kg per capita/year)						
Country	**1950s**	**1960s**	**1970s**	**1980s**	**1990s**	**2000s**	**National ban[b]**
Americas, excluding South America							
Canada	2.76	3.46	4.37	2.74	1.96	0.32	No ban
Cuba	ND	ND	ND	0.15	0.36	0.74	No ban
Mexico	0.28	0.57	0.97	0.77	0.39	0.26	No ban
USA	3.82	3.32	2.40	0.77	0.08	0.01	No ban
Others[c] (n = 12)	0.06	0.22	0.44	0.29	0.07	0.07	0/12
South America							
Argentina	ND	0.88	0.76	0.40	0.18	0.04	2001
Brazil	0.27	0.38	0.99	1.25	1.07	0.74	2001
Chile	0.07	0.92	0.56	0.64	0.55	0.03	2001
Ecuador	ND	ND	0.67	0.52	0.14	0.26	No ban
Uruguay	ND	0.74	0.75	0.54	0.47	0.08	2002
Others[c] (n = 6)	0.27	0.43	0.60	0.47	0.29	0.19	0/6
Oceania							
Australia	3.24	4.84	5.11	1.82	0.09	0.03	2003
New Zealand	2.05	2.56	2.90	1.00	ND	ND	No ban
Others[c] (n = 3)	ND	ND	ND	ND	ND	0.22	0/3

[a] Numbers corresponding to use of asbestos by country and region were calculated as annual use per capita averaged over the respective decade.
[b] Year first achieved or year planned to achieve ban. When shown as fraction, the numerator is the number of countries that achieved bans and the denominator is the number of other countries in the region.
[c] Data on asbestos use were available (but mortality data unavailable) for others in each region, in which case data were aggregated.
ND, no data available; NA, not applicable because of negative use data; 0.00 when the calculated data were < 0.005; 0 if there are no data after the year the ban was introduced.
From Nishikawa et al. (2008)

1.4.3 Water

Asbestos can enter the aquatic environment from both natural and anthropogenic sources, and has been measured in both ground- and surface-water samples. Erosion of asbestos-bearing rock is the principal natural source. Anthropogenic sources include: erosion of waste piles containing asbestos, corrosion of asbestos-cement pipes, disintegration of asbestos-containing roofing materials, and, industrial wastewater run-off (ATSDR, 2001).

1.4.4 Soil

Asbestos can enter the soil and sediment through natural (e.g. weathering and erosion of asbestos-bearing rocks) and anthropogenic (e.g. disposal of asbestos-containing wastes in landfills) sources. The practice of disposing asbestos-containing materials in landfills was more common in the past, and is restricted in many countries by regulation or legislation (ATSDR, 2001).

1.4.5 Environmental releases

According to the US EPA Toxics Release Inventory, total releases of friable asbestos to the environment (includes air, water, and soil) in 1999 were 13.6 million pounds from 86 facilities that reported producing, processing, or using asbestos (ATSDR, 2001). In 2009, total releases of 8.9 million pounds of friable asbestos were reported by 38 facilities (US EPA, 2010).

1.5 Human exposure

Inhalation and ingestion are the primary routes of exposure to asbestos. Dermal contact is not considered a primary source, although it may lead to secondary exposure to fibres, via ingestion or inhalation. The degree of penetration in the lungs is determined by the fibre diameter, with thin fibres having the greatest potential for deep lung deposition (NTP, 2005).

1.5.1 Exposure of the general population

Inhalation of asbestos fibres from outdoor air, and to a lesser degree in indoor air, is the primary route of exposure for the non-smoking general population. Exposure may also occur via ingestion of drinking-water, which has been contaminated with asbestos through erosion of natural deposits, erosion of asbestos-containing waste sites, corrosion of asbestos-containing cement pipes, or filtering through asbestos-containing filters. Families of asbestos-workers may be exposed via contact with fibres carried home on hair or on clothing.

In studies of asbestos concentrations in outdoor air, chrysotile is the predominant fibre detected. Low levels of asbestos have been measured in outdoor air in rural locations (typical concentration, 10 fibres/m^3 [f/m^3]). Typical concentrations are about 10-fold higher in urban locations and about 1000 times higher in close proximity to industrial sources of exposure (e.g. asbestos mine or factory, demolition site, or improperly protected asbestos-containing waste site) (ATSDR, 2001). Asbestos fibres (mainly chrysotile) were measured in air and in settled dust samples obtained in New York City following destruction of the World Trade Center on September 11, 2001 (Landrigan *et al.*, 2004).

In indoor air (e.g. in homes, schools, and other buildings), measured concentrations of asbestos are in the range of 30–6000 f/m^3. Measured concentrations vary depending on the application in which the asbestos was used (e.g. insulation versus ceiling or floor tiles), and on the condition of the asbestos-containing materials (i.e. good condition versus deteriorated and easily friable) (ATSDR, 2001).

1.5.2 Occupational exposure

Asbestos has been in widespread commercial use for over 100 years (USGS, 2001). Globally, each year, an estimated 125 million people are occupationally exposed to asbestos (WHO, 2006). Exposure by inhalation, and to a lesser extent ingestion, occurs in the mining and milling of asbestos (or other minerals contaminated with asbestos), the manufacturing or use of products containing asbestos, construction, automotive industry, the asbestos-abatement industry (including the transport and disposal of asbestos-containing wastes).

Estimates of the number of workers potentially exposed to asbestos in the USA have been reported by the National Institute of Occupational Safety and Health (NIOSH), by the Occupational Safety and Health Administration (OSHA), and the Mine Safety and Health Administration (MSHA). OSHA estimated in 1990 that about 568000 workers in production and services industries and 114000 in construction industries may have been exposed to asbestos in the workplace (OSHA, 1990). Based on mine employment data from 2002, NIOSH estimated that 44000 miners and other mine workers may have been exposed to asbestos during the mining of asbestos and some mineral commodities in which asbestos may have been a potential contaminant (NIOSH, 2002b). More recently, OSHA has estimated that 1.3 million employees in construction and general industry face significant asbestos exposure on the job (OSHA, 2008). In addition to evidence from OSHA and MSHA that indicate a reduction in occupational exposures in the USA over the past several decades, other information compiled on workplace exposures to asbestos

indicates that the nature of occupational exposures to asbestos has changed (Rice & Heineman, 2003). Once dominated by chronic exposures in manufacturing process such as textile mills, friction-product manufacturing, and cement-pipe fabrication, current occupational exposures to asbestos primarily occur during maintenance activities or remediation of buildings that contain asbestos.

In Europe, estimates of the number of workers exposed to asbestos have been developed by CAREX (CARcinogen EXposure). Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that a total of 1.2 million workers were exposed to asbestos in 41 industries in the 15 Member States of the EU. Over 96% of these workers were employed in the following 15 industries: 'construction' (n = 574000), 'personal and household services' (n = 99000), 'other mining' (n = 85000), 'agriculture' (n = 81000), 'wholesale and retail trade and restaurants and hotels' (n = 70000), 'food manufacturing' (n = 45000), 'land transport' (n = 39000), 'manufacture of industrial chemicals' (n = 33000), 'fishing' (n = 25000), 'electricity, gas and steam' (n = 23000), 'water transport' (n = 21000), 'manufacture of other chemical products' (n = 19000), 'manufacture of transport equipment' (n = 17000), 'sanitary and similar services' (n = 16000), and 'manufacture of machinery, except electrical' (n = 12000). Despite the total ban of asbestos, about 1500 workers (mainly construction workers and auto mechanics) were reported as having exposure to asbestos on the Finnish Register of Workers Exposed to Carcinogens (ASA Register) in 2006 (Saalo *et al.*, 2006). In 2004, approximately 61000 workers performing demolition and reconstruction work in Germany were registered in the Central Registration Agency for Employees Exposed to Asbestos Dust (Hagemeyer *et al.*, 2006).

Exposure to asbestos in occupational settings is regulated in countries of the EU. According to the European Directive of the EC 2003/18, permissible limits are 0.1 [f/mL] for all types of asbestos, based on an 8-hour time-weighted average (8h-TWA) (EU, 2003). The same limit is in force in most Canadian provinces (Alberta, British Columbia, Manitoba, Ontario, Newfoundland and Labrador, Prince Edward Island, New Brunswick and Nova Scotia); New Zealand; Norway; and, the USA. Other countries have permissible limits of up to 2 fibres/cm^3 (ACGIH, 2007).

Since 1986, the annual geometric means of occupational exposure concentrations to asbestos reported in the OSHA database and the MSHA database have been consistently below the NIOSH recommended exposure limit (REL) of 0.1 f/mL for all major industry divisions in the USA. The number of occupational asbestos exposure samples that were measured and reported by OSHA decreased from an average of 890 per year during 1987–94 to 241 per year during 1995–99. The percentage exceeding the NIOSH REL decreased from 6.3% during 1987–1994 to 0.9% during 1995–99. During the same two periods, the number of exposures measured and reported in the MSHA database decreased from an average of 47 per year during 1987–94 to an average of 23 per year during 1995–99. The percentage exceeding the NIOSH REL decreased from 11.1% during 1987–94 to 2.6% during 1995–99 (NIOSH, 2002a).

Data from studies and reviews of occupational asbestos exposure published since the previous *IARC Monograph* (IARC, 1973) are summarized below.

(a) Studies of occupational exposure

In a mortality study of 328 employees of an asbestos-cement factory in Ontario, Canada, Finkelstein (1983) constructed an exposure model on the basis of available air sampling data, and calculated individual exposure histories to

investigate exposure–response relationships for asbestos-associated malignancies. In retrospectively estimating exposure, the following assumptions were made: exposures did not change during 1962–70, exposures during 1955–61 were 30% higher than the later period, and exposures during 1948–54 were twice as high as during 1962–70. Exposure estimates for the years 1949, 1969, and 1979 were as follows: 40, 20, 0.2 f/mL for the willows operators; 16, 8, 0.5 f/mL for the forming machine operators; and, 8, 4, 0.3 f/mL for the lathe operators.

In an occupational hygiene survey of 24 Finnish workplaces, asbestos concentrations were measured during the different operations of brake maintenance of passenger cars, trucks and buses. During brake repair of trucks or buses, the estimated 8-hour time-weighted average exposure to asbestos was 0.1–0.2 [f/mL]. High levels of exposure (range, 0.3–125 [f/mL]; mean, 56 [f/mL]) were observed during brake maintenance if local exhaust ventilation was not used. Other operations in which the concentration exceeded 1 [f/mL] included cleaning of brakes with a brush, wet cloth or compressed air jet without local exhaust (Kauppinen & Korhonen, 1987).

Kimura (1987), in Japan, reported the following geometric mean concentrations: bag opening and mixing, 4.5–9.5 f/mL in 1970–75 and 0.03–1.6 f/mL in 1984–86; cement cutting and grinding, 2.5–3.5 f/mL in 1970–75 and 0.17–0.57 f/mL in 1984–86; spinning and grinding of friction products, 10.2–35.5 f/mL in 1970–75 and 0.24–5.5 f/mL in 1984–86.

Albin *et al.* (1990) examined total and cause-specific mortality among 1929 Swedish asbestos cement workers employed at a plant producing various products (e.g. sheets, shingles, ventilation pipes) from chrysotile and, to a lesser extent, crocidolite and amosite asbestos. Individual exposures were estimated using dust measurements available for the period 1956–77. Levels of exposure were estimated for the following operations: milling, mixing, machine line, sawing, and grinding. Asbestos concentrations ranged from 1.5–6.3 f/mL in 1956, to 0.3–5.0 f/mL in 1969, and to 0.9–1.7 f/mL in 1975. In all three time periods, the highest concentrations were observed in the milling and grinding operations.

The Health Effects Institute (1991) evaluated an operation and maintenance programme in a hospital on the basis of 394 air samples obtained during 106 on-site activities. The mean asbestos concentration was approximately 0.11 f/mL for personal samples, and approximately 0.012 f/mL for area samples. Eight-hour TWA concentrations showed that 99% of the personal samples were below 0.2 f/mL, and 95% below 0.1 f/mL.

Price *et al.* (1992) estimated the TWAs of asbestos exposures experienced by maintenance personnel on the basis of 1227 air samples collected to measure airborne asbestos levels in buildings with asbestos-containing materials. TWA exposures were 0.009 f/mL for telecommunication switch work, 0.037 f/mL for above-ceiling maintenance work, and 0.51 f/mL for work in utility spaces. Median concentrations were in the range of 0.01–0.02 f/mL.

Weiner *et al.* (1994) reported concentrations in a South African workshop in which chrysotile asbestos cement sheets were cut into components for insulation. The sheets were cut manually, sanded and subsequently assembled. Initial sampling showed personal sample mean concentrations of 1.9 f/mL for assembling, 5.7 f/mL for sweeping, 8.6 f/mL for drilling, and 27.5 f/mL for sanding. After improvements and clean-up of the work environment, the concentrations fell to 0.5–1.7 f/mL.

In a 1985 study, Higashi *et al.* (1994) collected personal and area samples at two manufacturing and processing locations in five Japanese plants manufacturing asbestos-containing products (a roofing material plant; a plant making asbestos cement sheets; a friction-material plant; and two construction and roofing-material plants). Geometric average concentrations of 0.05–0.45

f/mL were measured in area samples, and 0.05–0.78 f/mL in personal samples.

To assess the contribution of occupational asbestos exposure to the occurrence of mesothelioma and lung cancer in Europe, Albin *et al.* (1999) reviewed and summarized the available information on asbestos consumption in Europe, the proportion of the population exposed and levels of exposure. Ranges of exposure were reported for the former Yugoslavia, Poland, and Latvia. In 1987, mean fibre concentrations in Serbia and Montenegro were 2–16 f/mL for textile manufacturing, 3–4 f/mL for friction materials production, and 1–4 f/mL for asbestos cement production. In Poland, exposure levels in 1994 were estimated to be much greater than 2 f/mL in the textile industry, approximately 2 f/mL in asbestos cement and friction-products manufacturing, and greater than 0.5 f/mL in downstream use. In the Latvian asbestos cement industry in 1994, ranges of fibre concentrations were 0.1–1.1 f/mL for the machine line, and 1.1–5.2 f/mL for the milling and mixing areas.

Since 1974, NIOSH has conducted a series of sampling surveys in the USA to gather information on exposure of brake mechanics to airborne asbestos during brake repair. These surveys indicated that the TWA asbestos concentrations (about 1–6 hours in duration) during brake servicing were in the range of 0.004–0.28 f/mL, and the mean TWA concentration, approxiamtely 0.05 f/mL (Paustenbach *et al.*, 2004).

Based on a review of the historical literature on asbestos exposure before 1972 and an analysis of more than 26000 measurements collected during 1972–90, Hagemeyer *et al.* (2006) observed a continual decrease in workplace levels of airborne asbestos from the 1950s to 1990 in Western Germany (FRG) and Eastern Germany (GDR). High concentrations of asbestos fibres were measured for some working processes in Western Germany (e.g. asbestos spraying (400 [f/mL]), removal of asbestos insulations in the ship repair industry (320 [f/mL]), removal of asbestos insulation (300 [f/mL]), and cutting corrugated asbestos sheets (60 [f/mL]), see Table 1.3.

In a study at a large petroleum refinery in Texas, USA, Williams *et al.* (2007a) estimated 8h-TWA asbestos exposures for 12 different occupations (insulators, pipefitters, boilermakers, masons, welders, sheet-metal workers, millwrights, electricians, carpenters, painters, laborers, and maintenance workers) from the 1940s to the 1985 onwards. Estimates were calculated using information on the historical use of asbestos, the potential for exposure due to daily work activities, occupational hygiene sampling data, historical information on task-specific exposures, and use of personal protective equipment. Exposures were estimated for 1940–50, 1951–65, 1966–71, 1972–75, 1976–85, and 1985 onwards. For these time periods, the 8h-TWA exposure (50^{th} percentile) estimates for insulators were, respectively, 9 f/mL, 8 f/mL, 2 f/mL, 0.3 f/mL, 0.005 f/mL, and < 0.001 f/mL. For all other occupations, with the exception of labourers, estimated 8h-TWA exposure estimates were at least 50- to 100-fold less than that of insulators. Estimated 8h-TWA exposure estimates for labourers were approximately one-fifth to one-tenth of those of insulators.

Williams *et al.* (2007b) reviewed historical asbestos exposures (1940–2006) in various non-shipyard and shipyard settings for the following skilled occupations: insulators, pipefitters, boilermakers, masons, welders, sheet-metal workers, millwrights, electricians, carpenters, painters, labourers, maintenance workers, and abatement workers. For activities performed by insulators in various non-shipyard settings from the late 1960s and early 1970s, average task-specific and/or full-shift airborne fibre concentrations ranged from about 2 to 10 f/mL. Average fibre concentrations in US shipyards were about 2-fold greater, and excessively high concentrations (attributed to the spraying of asbestos) were reported in some British Naval shipyards. The introduction of improved occupational hygiene

Table 1.3 Examples of asbestos fibre concentrations in the air (f/cm³) of different workplaces in Germany

Work area		1950–54[a]	1970–74	1980	1990
Textile industries	FRG	100	10	3.8	0.9
	GDR	100	12	6.2	2.2
Production of gaskets	FRG	60	6.6	4.7	0.7
	GDR	60	8.0	7.8	1.6
Production of cement	FRG	200	11	1.1	0.3
	GDR	200	13	1.9	0.7
Production of brake pads	FRG	150	9.1	1.4	0.7
	GDR	150	11	2.4	1.6
Insulation works	FRG	15	15	8.6	0.2
	GDR	18	18	14.0	0.5

[a] Data for the GDR before 1967 are extrapolated
FRG, Federal Republic of Germany; GDR, German Democratic Republic
From Hagemeyer et al. (2006)

practices resulted in a 2- to 5-fold reduction in average fibre concentrations for insulator tasks. The typical range of average fibre concentration for most other occupations was < 0.01–1 f/mL. Concentrations varied with task and time period, with higher concentrations observed for tasks involving the use of powered tools, the mixing or sanding of drywall cement, and the cleanup of asbestos insulation or lagging materials. It was not possible with the available data to determine whether the airborne fibres were serpentine or amphibole asbestos.

Madl *et al.* (2007) examined seven simulation studies and four work-site industrial hygiene studies to estimate the concentration of asbestos fibres to which workers may have historically been exposed while working with asbestos-containing gaskets and packing materials in specific industrial and maritime settings (e.g. refinery, chemical, ship/shipyard). These studies involved the collection of more than 300 air samples and evaluated specific activities, such as the removal and installation of gaskets and packings, flange cleaning, and gasket formation. In all but one of the studies, the short-term average exposures were less than 1 f/mL, and all of the long-term average exposures were less than 0.1 f/mL. Higher short-term average concentrations were observed during the use of powered tools versus hand-held manual tools during gasket formation (0.44 f/mL versus 0.1 f/mL, respectively). Peak concentrations of 0.14 f/mL and 0.40 f/mL were observed during 'gasket removal and flange face cleaning with hand tools' and 'packing removal and installation', respectively.

(b) Dietary exposure

The general population can be exposed to asbestos in drinking-water. Asbestos can enter potable water supplies through the erosion of natural deposits or the leaching from waste asbestos in landfills, from the deterioration of asbestos-containing cement pipes used to carry drinking-water or from the filtering of water supplies through asbestos-containing filters. In the USA, the concentration of asbestos in most drinking-water supplies is less than 1 f/mL, even in areas with asbestos deposits or with asbestos cement water supply pipes. However, in some locations, the concentration in water may be extremely high, containing 10–300 million f/L (or even higher). The average person drinks about 2 litres of water per day (ATSDR, 2001). Risks of exposure to asbestos in drinking-water

may be especially high for small children who drink seven times more water per day per kg of body weight than the average adult (National Academy of Sciences, 1993).

1.6 Talc containing asbestiform fibres

Talc particles are normally plate-like. These particles, when viewed on edge under the microscope in bulk samples or on air filters, may appear to be fibres, and have been misidentified as such. Talc may also form true mineral fibres that are asbestiform in habit. In some talc deposits, tremolite, anthophyllite, and actinolite may occur. Talc containing asbestiform fibres is a term that has been used inconsistently in the literature. In some contexts, it applies to talc containing asbestiform fibres of talc or talc intergrown on a nanoscale with other minerals, usually anthophyllite. In other contexts, the term asbestiform talc has erroneously been used for talc products that contain asbestos. Similarly, the term asbestiform talc has erroneously been used for talc products that contain elongated mineral fragments that are not asbestiform. These differences in the use of the same term must be considered when evaluating the literature on talc. For a more detailed evaluation of talc not containing asbestiform fibres, refer to the previous *IARC Monograph* (IARC, 2010).

1.6.1 Identification of the agent

Talc (CAS No. 14807-96-6) is a designation for both the mineral talc and for commercial products marketed as 'talc', which contain the mineral in proportions in the range of 35% to almost 100%. Commercial talc is classified as 'industrial talc' (refers to products containing minerals other than talc), 'cosmetic talc' (refers to products, such as talcum powder, which contain > 98% talc), and 'pharmaceutical talc' (refers to products containing > 99% talc) (Rohl *et al.*, 1976; Zazenski *et al.*, 1995). Synonyms for talc include: Agalite, French chalk, kerolite, snowgoose, soapstone, steatite, talcite, and talcum.

1.6.2 Chemical and physical properties of the agent

The molecular formula of talc is $Mg_3Si_4O_{10}(OH)_2$. It is a hydrated magnesium sheet silicate mineral, whose structure is composed of a layer of $MgO_4(OH)_2$ octahedra sandwiched between identical layers of SiO_4 tetrahedra. In nature, the composition of talc varies depending on whether or not the magnesium has been substituted with other cations, such as iron, nickel, chromium or manganese (Rohl *et al.*, 1976; IMA, 2005). Pure talc is translucent, appearing white when finely ground (Zazenski *et al.*, 1995). The colour of talc changes in the presence of substituted cations, ranging from pale-green to dark-green, brownish or greenish-grey. Talc has the following chemical and physical properties: melting point, 1500°C; hardness, 1 on the Moh's scale of mineral hardness; density, 2.58–2.83; and cleavage, (001) perfect (Roberts *et al.*, 1974). Talc is a very stable mineral, and is insoluble in water, weak acids and alkalis, is neither explosive nor flammable, and has very little chemical reactivity (IMA, 2005).

Talc's structure is crystalline. It can have a small, irregular plate structure (referred to as microcrystalline talc) or it can have large, well defined platelets (referred to as macrocrystalline talc). Its platyness and crystallinity determine the specific commercial applications for which it is suitable (Zazenski *et al.*, 1995). Talc is formed by complex geological processes acting on pre-existing rock formations with diverse chemical composition (Rohl *et al.*, 1976). Many talc-bearing rocks are formed from magnesia- and silica-rich ultramafic rocks. These rocks have a central core of serpentinite surrounded by successive shells of talc-abundant rock (e.g. talc carbonate and steatite). The serpentinite core is composed mostly of non-asbestiform serpentine minerals (lizardite

and antigorite); however, small amounts of chrysotile asbestos may occur. (Zazenski *et al.*, 1995).

More detail on the chemical and physical properties of talc can be found in the previous *IARC Monograph* (IARC, 2010).

1.6.3 Use of the agent

Talc has several unique chemical and physical properties (such as platyness, softness, hydrophobicity, organophilicity, inertness) that make it desirable for a wide range of industrial and commercial applications (e.g. paint, polymers, paper, ceramics, animal feed, rubber, roofing, fertilizers, and cosmetics). In these products, talc acts as an anti-sticking and anti-caking agent, lubricant, carrier, thickener, absorbent, and strengthening and smoothing filler (IMA, 2005).

In 2000, the worldwide use pattern for talc was as follows: paper industry, 30%; ceramics manufacture, 28%; refractories, 11%; plastics, 6%; filler or pigment in paints, 5%; roofing applications, 5%; cement, 3%; cosmetics, 2%; and other miscellaneous uses, 10% (includes agriculture and food, art sculpture, asphalt filler, autobody filler, construction caulks, flooring, and joint compounds) (Roskill Information Services Ltd, 2003). According to a Mineral Commodity Summary published by the USGS in 2009, talc produced in the USA was used for ceramics, 31%; paper, 21%; paint, 19%; roofing, 8%; plastics, 5%; rubber, 4%; cosmetics, 2%; and other, 10% (Virta, 2009).

No information on the use of asbestiform talc in various industries (apart from mining and milling of talc from deposits containing asbestiform fibres) was identified by the Working Group. For a more detailed description of the uses of talc, refer to the previous *IARC Monograph* (IARC, 2010).

1.6.4 Environmental occurrence

(a) Natural occurrence

Primary talc deposits are found in almost every continent around the world. Talc is commonly formed by the hydrothermal alteration of magnesium- and iron-rich rocks (ultramafic rocks) and by low-grade thermal metamorphism of siliceous dolomites (Zazenski *et al.*, 1995). For more detailed information on the formation of commercially important talc deposits, refer to the previous *IARC Monograph* (IARC, 2010).

Talc deposits whose protoliths are ultramafic rocks (or mafic) are abundant in number but small in total production. They are found in discontinuous bodies in orogenic belts such as the Alps, the Appalachians, and the Himalayas; these types of talc deposits form during regional metamorphism accompanying orogenesis. They also occur in the USA (California, Arkansas, Texas), Germany, Norway, Canada (Ontario and Quebec), southern Spain, Finland, the Russian Federation (Shabry and Miassy), and Egypt. Chlorite and amphibole are usually associated with this type of talc deposit although they are commonly separated in space from the talc ore (Vermont). The amphiboles may or may not be asbestiform, depending on the local geological history (IARC, 2010).

Talc deposits formed from the alteration of magnesian carbonate and sandy carbonate such as dolomite and limestone are the most important in terms of world production. Two types are recognized:

- those derived from hydrothermal alteration of unmetamorphosed or minimally metamorphosed dolomite such as found in Australia (Mount Seabrook and Three Springs); USA (Wintersboro, Alabama; Yellowstone, Montana; Talc City, California; Metaline Falls, Washington; and West Texas); the Republic of Korea; the People's Republic of China; India; the

Russian Federation (Onot); and, northern Spain (Respina)

- those derived from hydrothermal alteration (including retrograde metamorphism) of regionally metamorphosed siliceous dolomites and other magnesium-rich rocks such as in the USA (Murphy Marble belt, North Carolina; Death Valley-Kingston Range, California; Gouverneur District, New York; Chatsworth, Georgia); Canada (Madoc); Italy (Chisone Valley); the Russian Federation (Krasnoyarsk); Germany (Wunsiedel); Austria (Leoben); Slovakia (Gemerska); Spain; France (Trimouns); and Brazil (Brumado) (IARC, 2010).

In a study to examine the amphibole asbestos content of commercial talc deposits in the USA, Van Gosen *et al.* (2004) found that the talc-forming environment (e.g. regional metamorphism, contact metamorphism, or hydrothermal processes) directly influenced the amphibole and amphibole-asbestos content of the talc deposit. Specifically, the study found that hydrothermal talcs consistently lack amphiboles as accessory minerals, but that contact metamorphic talcs show a strong tendency to contain amphiboles, and regional metamorphic talc bodies consistently contain amphiboles, which display a variety of compositions and habits (including asbestiform). Death Valley, California is an example of a contact metamorphic talc deposit that contains accessory amphibole-asbestos (namely talc-tremolite).

1.6.5 Human exposure

(a) Exposure of the general population

Consumer products (e.g. cosmetics, pharmaceuticals) are the primary sources of exposure to talc for the general population. Inhalation and dermal contact (i.e. through perineal application of talcum powders) are the primary routes of exposure. As talc is used as an anti-sticking agent in several food preparations (e.g. chewing gum), ingestion may also be a potential, albeit minor, route of exposure.

As late as 1973, some talc products sold in the USA contained detectable levels of chrysotile asbestos, tremolite, or anthophyllite (Rohl *et al.*, 1976), and it is possible that they remained on the market in some places in the world for some time after that (Jehan, 1984). Some of the tremolite and anthophyllite may have been asbestiform in habit (Van Gosen, 2006).

Blount (1991) examined pharmaceutical- and cosmetic-grade talcs for asbestiform amphibole content using a density-optical method. High-grade talc product samples (n = 15) were collected from deposits in Montana, Vermont, North Carolina, Alabama, and from outside the USA but available in the US market. Samples were uniformly low in amphibole content (with counts in the range of 0–341 particles/mg), and some samples appeared to be completely free of amphibole minerals. In samples containing amphibole minerals, cleavage-type and asbestos-type minerals were observed. Only one sample was found to contain an amphibole particle size distribution typical of asbestos.

More complete information on the levels of exposure experienced by the general population can be found in the previous *IARC Monograph* (IARC, 2010).

(b) Occupational exposure

Inhalation is the primary route of exposure to talc in occupational settings. Exposure by inhalation to talc dust occurs in the talc-producing industries (e.g. during mining, crushing, separating, bagging, and loading), and in the talc-using industries (e.g. rubber dusting and addition of talcs to ceramic clays and glazes). Because industrial talc is a mixture of various associated minerals, occupational exposure is to a mixture of mineral dusts (IARC, 1987b).

In general, data on numbers of workers occupationally exposed to talc are lacking. The

National Occupation Exposure Survey (NOES), which was conducted by the US National Institute for Occupational Safety and Health (NIOSH) during 1981–83, estimated that 1.4 million workers, including approximately 350000 female workers, were potentially exposed to talc in the workplace (NIOSH, 1990). CAREX reports that approximately 28000 workers were exposed to talc containing asbestiform fibres in the workplace within the 15 countries that comprised the EU during 1990–93; however, some major industries producing or using talc were not included.

Many of the early measurements reported very high levels of talc dust exposures in mining and milling operations, often in the range of several mg/m^3, but there is evidence of decreasing exposures (IARC, 1987b; IARC, 2010). For example, before the adoption of technical preventive means in 1950, exposures in the talc operation in the Germanasca and Chisone Valley (Piedmont), Italy, were reported to be approximately 800 mppcf in the mines, and approximately 25 mppcf in the mills. Exposures in both areas were reduced to less than 10 mppcf after 1965 when improved occupational hygiene practices were implemented (Rubino *et al.*, 1976). Although the presence of asbestiform talc was often not reliably verified, it is likely that these levels have also decreased, in part due to mine closures and regulatory controls.

Oestenstad *et al.* (2002) developed a job–exposure matrix for respirable dust, covering all work areas in an industrial grade (tremolitic) talc mining and milling facility in upstate New York, USA. The facility started operating in 1948 with the opening of an underground mine (Mine 1) and a mill (Mill 1). An open pit mine (Mine 2) opened in 1974. Talc from the facility was used predominantly for manufacturing paint and ceramic tiles. The range of all respirable dust concentrations measured in the two baseline exposure surveys was 0.01–2.67 mg/m^3, with an arithmetic mean of 0.47 mg/m^3 and a geometric mean of 0.28 mg/m^3.

Only limited information is available about exposures in secondary industries in which talc is used or processed further. The previous *IARC Monograph* on talc (IARC, 2010) summarizes three historical surveys conducted in these kinds of industries. The IARC Working Group in 1987 noted, however, that even when measurements of respirable fibres were reported, no electron microscopic analysis was conducted to confirm the identity of the fibres. Recently, most industries using talc use non-asbestiform talc (IARC, 2010).

For a more complete description of studies in which occupational exposure to talc and talc-containing products has been reported, refer to the previous *IARC Monograph* (IARC, 2010).

2. Cancer in Humans

2.1 Introduction

The previous *IARC Monographs* were limited to the same six commercial forms of asbestos fibres (chrysotile, actinolite, amosite, anthophyllite crocidolite and tremolite) that are subject of this current evaluation. In the previous *IARC Monograph* (IARC, 1977), the epidemiological evidence showed a high incidence of lung cancer among workers exposed to chrysotile, amosite, anthophyllite, and with mixed fibres containing crocidolite, and tremolite. Pleural and peritoneal mesotheliomas were reported to be associated with occupational exposures to crocidolite, amosite, and chrysotile. Gastrointestinal tract cancers were reported to have been demonstrated in groups occupationally exposed to amosite, chrysotile or mixed fibres containing chrysotile. An excess of cancer of the larynx in occupationally exposed individuals was also noted. Finally the *Monograph* points out that mesothelioma may occur among individuals living in neighbourhoods of asbestos factories

and crocidolite mines, and in persons living with asbestos workers.

Extensive epidemiological research on asbestos has been conducted since then. The associations between asbestos exposure, lung cancer, and mesothelioma have been well established in numerous epidemiological investigations. The epidemiological evidence for other cancer sites is less extensive than it is for lung cancer and mesothelioma, but is still considerable for some. In reviewing these studies, there are some common limitations that need to be borne in mind, which may explain the heterogeneity of the findings from the studies such as:

- The types, fibre sizes and levels of asbestos exposure differed from industry to industry and over time. Most of the heaviest exposures probably occurred in the first two-thirds of the twentieth century in asbestos mining and milling, insulation work, shipyard work, construction, and asbestos textile manufacture. Workers in different industries, eras, and geographic locales were exposed to different types of asbestos fibres, and to fibres of greatly varying dimensions.
- There were differences in how the studies handle the issue of latency or in other words time since first occupational exposure to asbestos. Some studies, especially earlier investigations, accumulated person–years from first exposure, a procedure that may dilute observed risk by including many years of low risk. Others have only accumulated person–years after a certain period of time after first exposure, usually 20 years. Also different studies followed their populations for very different periods of time since first occupational exposure to asbestos.
- The most pervasive problem in interpreting studies was the wide variation among studies in the approaches taken for exposure assessment. Some studies made no attempt to assess exposure beyond documenting employment of study participants in a trade or industry with potential for occupational exposure to asbestos. Other studies used surrogate indices of exposure such as duration of employment or self-reported intensity of exposure, or stratified subjects' exposure by job title. Some used the skills and knowledge of industrial hygienists, obtained direct measurements of asbestos dust levels in air, and developed job–exposure matrices and cumulative exposure indices. Even these analyses are limited by the fact that earlier studies used gravimetric measures of dust exposure, while later used fibre-counting methods based on phase contrast microscopy (PCM). Factors that were used to convert between gravimetric and PCM based measurements are generally unreliable unless they are based on side by side measurements taken in specific industrial operations. Differences in fibre size distributions and fibre type can only be detected using electron microscopy, which has been done in only a very few studies.
- Misclassification of disease was a serious problem for several of the cancer sites. This is particularly true for mesothelioma, which did not have diagnostic category in the ICD system until the 10th review was initiated in 1999.

There were also issues regarding the potential for misclassification of mesotheliomas as colon or ovarian cancers.

For talc that contains asbestiform fibres, previous Working Groups assessed studies on talc described as containing asbestiform tremolite and anthophyllite (IARC, 1987a, b). These fibres fit the definition of asbestos, and therefore a separate review of talc containing asbestiform fibres was not undertaken by this Working Group. The reader is invited to consult the General Remarks

in this volume for further details. For a review of Talc, refer to the previous *IARC Monograph* (IARC, 2010).

2.2 Cancer of the lung

2.2.1 Occupational exposure

Signs that cancer of the lung could be induced by exposure to asbestos was first raised by reports of lung cancer cases that occurred among workers with asbestosis (Gloyne, 1935; Lynch & Smith, 1935). The first cohort study that demonstrated an excess of lung cancer among asbestos exposed workers was a study of textile workers (Doll, 1955). In this study, 11 cases of lung cancer versus 0.8 expected ($P < 0.00001$) were reported based on national mortality rates. Since 1955, an association between lung cancer and occupational exposure to asbestos has been demonstrated in numerous cohort and case–control studies that are summarized in Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.1.pdf, Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.2.pdf, and Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.3.pdf.

Although a causal association between asbestos exposure and lung cancer is generally well recognized, there are still substantial controversies on how the risk might vary by exposure to different fibre types and sizes, and whether there is a risk at low levels of exposure (i.e. environmental exposures). Particularly controversial is the question of whether chrysotile asbestos is less potent for the induction of lung cancer than the amphibole forms of asbestos (e.g. crocidolite, amosite and tremolite), which has sometimes been referred to as the "amphibole hypothesis" (Cullen, 1996; Stayner *et al.*, 1996; McDonald, 1998). This argument is based on the observation from experimental studies that chrysotile asbestos is less biopersistent (i.e. has a shorter half life) in the lung than the amphiboles. Pathological studies of tissue using electron microscopy and energy dispersive analysis of X-rays (EDAX) have been used to measure the amounts of different asbestos fibre types in the lung. Case studies of Canadian chrysotile asbestos workers using these methods have shown an unexpectedly high proportion of amphibole (primarily tremolite) fibres, considering the relatively low percentage of amphibole fibres in commercial chrysotile asbestos (Pooley, 1976; Rowlands *et al.*, 1982; Addison & Davies, 1990). [The Working Group noted that the lower biopersistence of chrysotile in the lung does not necessarily imply that it would be less potent than amphiboles for lung cancer.]

Several meta-analyses have been conducted in which the relative potency of different fibre types and other fibre characteristics have been considered in relation to lung cancer. Lash *et al.* (1997) conducted a meta-analysis based on the findings from 15 cohort studies with quantitative information on the relationship between asbestos exposure and lung cancer risk. The slopes of the lung cancer exposure–response relationship from these studies were analysed using fixed and random effects models. Substantial heterogeneity in the slopes for lung cancer from these studies was found in their analysis. The heterogeneity was largely explained by industry category, dose measurements, tobacco habits, and standardization procedures. There was no evidence in this meta-analysis that differences in fibre type explained the heterogeneity of the slope.

Hodgson & Darnton (2000) performed a meta-analysis based on 17 cohort studies with information on the average level of asbestos exposure for the cohort as a whole or for subgroups in the study. The percentage excess lung cancer risk from each study or subgroup was divided by its average exposure level to derive a slope (RL) for the analysis. Substantial heterogeneity in the findings for lung cancer was also found in this

analysis particularly for the chrysotile cohorts. The heterogeneity in the findings for the chrysotile cohorts was largely attributable to differences in the findings from the studies of chrysotile miners and millers in Quebec (McDonald *et al.*, 1983), and asbestos textile workers in South Carolina (Dement & Brown, 1994; Hein *et al.*, 2007), which differed by nearly 100-fold. No explanation has been found for these extreme differences although several possible explanations have been investigated. Co-exposure to mineral oils in the South Carolina textile plant was proposed as a possible explanation. A nested case–control conducted with the South Carolina cohort failed to provide evidence to support the hypothesis that mineral exposure was associated with an increased risk of lung cancer in this study population (Dement & Brown, 1994). Differences in fibre size distributions have also been considered to be a potential explanation. The asbestos textile industry workers may have used a higher grade of asbestos resulting in exposures to a greater percentage of long fibres than what was experienced by miners and millers in Quebec. A larger percentage of long fibres was found in a recent reanalysis of samples from the South Carolina cohort using transmission electron microscopy (TEM) (Dement *et al.*, 2008) than what was previously reported in TEM analyses of samples from the Quebec mines and mills (Gibbs & Hwang, 1975, 1980). Based on their analysis, Hodgson & Darnton (2000) concluded that the ratio between lung cancer risk for chrysotile and the amphiboles was somewhere between 1:10 and 1:50. However, in their analyses (where they excluded the study of Quebec miners rather than the South Carolina cohort), there was only a 2-fold difference in findings for lung cancer risk between the chrysotile (RL = 2.3) and amphibole cohorts (RL = 4.2). [The Working Group noted that there is no justification for exclusion of the South Carolina cohort because it is one of the highest quality studies in terms of the exposure information used in this study.]

Berman & Crump (2008a) published a meta-analysis that included data from 15 asbestos cohort studies. Lung cancer risk potency factors (Kis = [RR-1]/cumulative exposure) were derived in their analyses that were specific for both fibre type (chrysotile versus amphiboles) and fibre size (length and width). Fibre size information was only available for one of the cohort studies, and for the other studies it was obtained from studies that were conducted in similar industrial settings. As with the previous analyses, substantial variation was found in the findings from these studies with results for lung cancer varying by two orders of magnitude, although no formal statistical tests of heterogeneity were performed. The hypothesis that chrysotile is equipotent as the amphiboles for lung cancer was not rejected for fibres of all widths ($P = 0.07$) or for thick (width > 0.2 μm) fibres ($P = 0.16$). For thin fibres (width < 0.2 μm), there was significant ($P = 0.002$) evidence that chrysotile fibres were less potent than amphiboles. Sensitivity analyses were also conducted in which the South Carolina or Quebec miners and millers cohorts were dropped from the analysis using fibres of all widths. Dropping the South Carolina cohort resulted in a highly significant ($P = 0.005$) result that potency was greater for amphiboles than for chrysotile. Dropping the Quebec cohort resulted in there being no significant ($P = 0.55$) evidence of a difference in potency between the fibre types. [The Working Group noted that both the Hodgson & Darnton and Berman & Crump analyses reveal a large degree of heterogeneity in the study findings for lung cancer, and that findings are highly sensitive to the inclusion or exclusion of the studies from South Carolina or Quebec. The reasons for the heterogeneity are unknown, and until they are explained it is not possible to draw any firm conclusions concerning the relative potency of chrysotile and amphibole asbestos fibres from these analyses.]

Based on findings from experimental studies, it is suspected that long and thin fibres are likely

to be more potent than short and thick fibres in the induction of lung cancer in humans. Unfortunately until recently, all of the epidemiological studies that have been conducted used methods for exposure assessment that did not include a determination of fibre size, and thus this issue could not be directly addressed with these studies. As described above, the meta-analysis conducted by Berman & Crump (2008a) considered the effect of fibre size on lung cancer risk by using data from other studies conducted in similar circumstances as the cohort studies. Their analysis did not reveal strong evidence that lung cancer potency was dependent on fibre size. There was weak evidence that long fibres (length > 10 μm) were more potent than short fibres (5 μm < length < 10 μm) in models using all widths ($P = 0.07$). The lack of size-specific data from the studies was a major limitation of this study with regard to estimating size-specific risk estimates. Stayner *et al.* (2008) published findings from an analysis of the South Carolina asbestos textile cohort in which fibre size specific estimates of lung cancer mortality was evaluated using information from a reanalysis of archived air samples using TEM (Dement *et al.*, 2008). Long fibres (> 10 μm) and thin fibres (< 0.25 μm) were found to be the strongest predictors of lung cancer mortality in this study.

Another study not part of the prior meta-analyses provides relevant information regarding the question of the relative lung cancer potency of the fibre types. Loomis *et al.* (2009) carried out a retrospective cohort mortality study of textile workers from four plants in North Carolina that had never been studied before. Workers in this cohort were primarily exposed to chrysotile asbestos that was imported from Quebec. A small amount of amosite was used in an operation in one of the plants. Overall, an excess of lung cancer was observed in this study (SMR, 1.96; 95%CI: 1.73–2.20), which was very similar in magnitude to that reported in the South Carolina cohort study of textile workers (Hein *et al.*, 2007). However, the slope for the exposure–response between asbestos exposure and lung cancer was considerably lower than that reported in the South Carolina cohort study. The reasons for these differences in the exposure–response relationships are unknown, but one possible reason may be that quality of the exposure information was superior in the South Carolina study, and that the difference could be explained by an attenuation of the slope due to exposure misclassification in Loomis *et al.* (2009).

2.2.2 Environmental exposures

Evidence of an association in women between lung cancer and environmental exposures in New Caledonia to field dust containing tremolite and the use of a whitewash ("po") containing tremolite has been reported (Luce *et al.*, 2000). A positive association with heavy residential exposure to asbestos was observed in a lung cancer case–control study the Northern Province of South Africa, which is a crocidolite and amosite mining area (Mzileni *et al.*, 1999). The association was strongest among women who resided in heavily exposed areas (odds ratio [OR], 5.4; 95%CI: 1.3–22.5; Ptrend = 0.02). A study of lung cancer mortality among women in two chrysotile mining regions of Quebec did not result in an increase in lung cancer (SMR, 0.99; 95%CI: 0.78–1.25) relative to women from 60 other areas of Canada (Camus *et al.*, 1998).

2.2.3 Non-commercial asbestiform amphibole fibres

There is emerging epidemiological evidence that non-commercial amphibole fibres that are asbestiform have carcinogenic potential. These fibres are not technically "asbestos," and they were never commercially marketed. However, the Working Group felt it was important to discuss the recent evidence concerning these

fibres because of their similarity to asbestos, and because of public concerns regarding this issue.

Several studies have described adverse health associations with the amphibole fibres that contaminated vermiculite mined in Libby, Montana, USA. These fibres were originally characterized as from the tremolite-actinolite series (IARC, 1987a), however, they have been more recently described by the US Geological Society as approximately 84% winchite, 11% richterite, and 6% tremolite (Meeker *et al.*, 2003). Sullivan (2007) reported standardized mortality ratios (SMRs), using cause of death data and expected mortality for the underlying cause of death based on national age-, race-, and sex-specific rates. Using a 15-year exposure lag, there were increased SMRs for all cancer (SMR, 1.4; 95%CI: 1.2–1.6; $n = 202$), and lung cancer (SMR, 1.7; 95%CI: 1.4–2.1; $n = 89$). Increasing risks were observed across categories of cumulative exposure; the SMR estimates were 1.5, 1.6, 1.8, and 1.9 in the 1–4.49, 4.5–22.9, 23.0–99.0, and ≥ 100 f/mL–years exposure categories, respectively. Results from other studies (Amandus *et al.*, 1987; McDonald *et al.*, 2004) of analyses using a continuous measure of exposure also resulted in statistically significant relationships with lung cancer mortality risk. For example, in the updated analysis by McDonald *et al.* (2004), the estimated linear increase in relative risk of respiratory cancer risk per 100 f/mL–years cumulative exposure was 0.36 (95%CI: 0.03–1.2; $P = 0.02$).

2.3 Mesothelioma

Pleural and peritoneal mesotheliomas are very rare malignancies that occur in the mesothelial cells that line these cavities. The first report of a possible association between asbestos exposure and mesothelioma was by Wagner *et al.* (1960) who described an outbreak of mesothelioma in a crocidolite mining region of South Africa. The majority of the cases reported had worked in the mines (23/33) but some of the cases had also occurred among individuals with no history of occupational exposures (10/33). Since then, an excess of mesothelioma has been observed in a large number of cohort and case–control studies (summarized in online Tables 2.2, 2.3 and Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.4.pdf) in a variety of different industries using and producing asbestos. Although the causal association between mesothelioma and asbestos has been well established, several important issues remain to be resolved that are discussed below.

2.3.1 Fibre type

Although all forms of asbestos can cause mesothelioma, there is considerable evidence that the potency for the induction of mesothelioma varies by fibre type, and in particular that chrysotile asbestos is less potent than amphibole forms of asbestos. An excess of mesothelioma has been reported in cohort studies of chrysotile exposed miners and millers in Quebec (Liddell *et al.*, 1997), and in South Carolina asbestos textile workers who were predominantly exposed to chrysotile asbestos imported from Quebec (Hein *et al.*, 2007). However, the fact that the chrysotile asbestos mined in Quebec is contaminated with a small percentage (< 1.0%) of amphibole (tremolite) asbestos has complicated the interpretation of these findings. McDonald *et al.* (1997) found in a nested case–control study for mesothelioma in the Thetford mines of Quebec that an association with asbestos exposure was evident in mines from a region with higher concentrations of tremolite, and not in another region with lower concentrations of tremolite. Bégin *et al.* (1992) noted that although tremolite levels may be 7.5 times higher in Thetford than in Asbestos, the incidence of mesothelioma in these two Quebec mining towns was proportional to the size of their workforce. This suggests that the tremolitic content of the ores may not be a

determinant of mesothelioma risk in Quebec. Separate analyses for workers at the Thetford and Asbestos mines and mills did not demonstrate a different exposure–response relationship for asbestos and mesothelioma in the two mining areas (McDonald & McDonald, 1995).

In a mesothelioma case–control study in South Africa, an association was reported with exposures to crocidolite and amosite asbestos, but no cases were found to have been exclusively exposed to chrysotile asbestos (Rees *et al.*, 1999). One possible explanation for these negative findings for chrysotile is that South African chrysotile asbestos may contain relatively little tremolite (Rees *et al.*, 1992). Another possible explanation is that chrysotile mining began later, and production levels were lower than in the crocidolite and amosite mines of South Africa. Cases of mesothelioma have been reported among asbestos miners in Zimbabwe, which has been reported to be uncontaminated with tremolite asbestos (Cullen & Baloyi, 1991). Excess mesothelioma mortality (standardized incidence ratio [SIR], 4.0, 95%CI: 1.5–8.7) was reported in miners and millers from a chrysotile mine in Balangero, Italy (Mirabelli *et al.*, 2008), reportedly free of amphibole contamination (Piolatto *et al.*, 1990).

An evaluation of the relative potency of the different fibre types of asbestos has been considered in the meta-analyses that were previously described (see prior section on lung cancer) by Hodgson & Darnton (2000) and Berman & Crump (2008a, b). Hodgson & Darnton (2000) used the percentage of mesothelioma deaths of all deaths expected (at an age of first exposure of 30) per unit of cumulative exposure (Rm) as the measure for their analysis. They computed separate estimates of Rm for crocidolite, amosite and chrysotile asbestos. Based on their analyses, they estimated that the ratio of the potency for mesothelioma (pleural and peritoneal combined) was 1:100:500 for chrysotile, amosite, and crocidolite respectively.

The meta-analysis conducted by Berman & Crump (2008a) was based on the analysis of the slopes (Km) that were estimated using an approach that assumes that the mortality rate from mesothelioma increases linearly with the intensity of exposure, and for a given intensity, increases indefinitely after exposure ceases, approximately as the square of time since first exposure (lagged 10 years). This model was tested with the raw data from several studies, and found to provide a good fit to the data (Berman & Crump, 2008b). Regression models were fitted to the study Km values that included information from surrogate studies to estimate fibre type (chrysotile versus amphiboles) and fibre length (short versus long) specific potency slopes (Berman & Crump, 2008a). Alternative models were fitted with exposure metrics based on different fibre widths. The hypothesis that chrysotile and amphibole forms of asbestos are equipotent was strongly rejected, and the hypothesis that potency for chrysotile asbestos was 0 could not be rejected based on their models ($P < 0.001$ and $P = 0.29$, respectively, for all-widths model). The best estimates for the relative potency of chrysotile ranged from zero to about 1/200th that of amphibole asbestos (depending on the width of the exposure metric used in the model). [The Working Group noted that there is a high degree of uncertainty concerning the accuracy of the relative potency estimates derived from the Hodgson & Darnton and Berman & Crump analyses because of the severe potential for exposure misclassification in these studies.]

Two newer studies, not part of the prior meta-analyses, provide important information regarding the question of the relative potency of the fibre types. The first is a study of a cohort of textile workers in North Carolina not previously examined (Loomis *et al.*, 2009). Workers in this cohort were primarily exposed to chrysotile asbestos imported from Quebec. A relatively large excess of both mesothelioma [SMR, 10.92; 95%CI: 2.98–27.96] and pleural cancer [SMR,

12.43; 95%CI: 3.39–31.83]. The pleural and mesothelioma deaths combined comprised 0.3% of all deaths. This percentage was nearly identical to the estimate developed for the chrysotile cohorts in a review article by Stayner *et al.* (1996). Based on the approach that Hodgson & Darnton used in their meta-analysis, the authors estimated that the percentage of deaths per unit of fibre exposure was 0.0058% per f–y/mL (0.0098% per f–y/mL for workers followed ≥ 20 years). This estimate was considerably higher than the estimate developed by Hodgson & Darnton of 0.0010% per f–yr/mL for cohorts exposed to chrysotile.

The other study investigated mesothelioma among chrysotile miners and millers, and resident communities in Balangero, Italy. The chrysotile mined at Balangero was reported to be free of tremolite and other amphiboles. The ore contains trace amounts of another fibre called blangeroite, which is not an amphibole (Turci *et al.*, 2009). A previous cohort of the miners and millers in Balangero with follow up to 1987 identified only two deaths from mesothelioma (Piolatto *et al.*, 1990). Cases of mesothelioma were identified from a local mesothelioma registry comprises people who had been mine employees; employees of subcontractors or other firms transporting or refining Balangero asbestos, asbestos ore; residents of the area who were exposed from air pollution, living with a mine employee or from mine tailings from Balangero. Six cases of mesothelioma were identified among blue-collar miners, and an estimated 1.5 deaths (SIR, 4.00; 95%CI: 1.47–8.71) would be expected based on a previous cohort study (Piolatto *et al.*, 1990), and conservative assumptions about the cohort. Additional cases of mesothelioma were identified among white-collar miners (three cases), workers in the mine hired by subcontractors (five cases), and from non-occupational exposures or exposure to re-used tailings (ten cases). Expected numbers of mesothelioma cases could not be derived for these groups because they were not part of the original cohort definition. The findings from this investigation indicate that the previous risk of mesothelioma for the Balangero cohort were seriously underestimated.

2.3.2 Fibre size

Based on a review of toxicological and human studies, Lippmann (1990) suggested that fibres shorter than 0.1 µm and longer than 5 µm are related to mesothelioma in humans. The Berman & Crump meta-analyses provided weak evidence that fibre length is a determinant of the potency of asbestos. The test of the hypothesis that long fibres (length ≥ 10 µm) and short fibres (5 < length < 10 µm) are equipotent was nearly rejected in some models (e.g. $P = 0.09$ for all widths). Thus, their findings provide weak support that long fibres may be more potent than short fibres for mesothelioma. There was little evidence in their analyses that thin fibres (width < 0.4 or < 0.2 µm) were stronger predictors of mesothelioma potency than all fibre widths combined. A major limitation of their analysis was that it relied on surrogate data to estimate the fibre-size distributions for the studies used in the meta-analysis.

2.3.3 Pleural versus peritoneal tumours

The ratio of pleural to peritoneal mesotheliomas has varied considerably in different epidemiological studies of asbestos-exposed cohorts. In the cohort studies included in the meta-analysis conducted by Hodgson & Darnton (2000), the percentage of mesotheliomas that were peritoneal varied from 0 to over 50%. Hodgson & Darnton reported that peritoneal mesotheliomas increased with the square of cumulative exposure to asbestos (i.e. a supralinear relationship); whereas pleural mesotheliomas increased less than linearly with cumulative exposure to asbestos. This implies that the number of peritoneal mesotheliomas would dramatically increase relative to the number of pleural mesotheliomas at high asbestos exposure levels. Welch *et al.*

(2005) found a strong association (OR, 5.0; 95%CI: 1.2–21.5) between asbestos exposure and peritoneal cancer in a population-based case–control study. This study included a large percentage of men with what were judged to be low exposures to asbestos.

2.3.4 Environmental exposures

An excess of mesothelioma has been observed in several studies of communities with environmental exposure to asbestos. A large excess of mesothelioma was reported in a study of people living in villages in Turkey exposed to erionite used to whitewash their homes (Baris *et al.*, 1987). An excess in mesothelioma was reported among people living near crocidolite mining regions in South Africa and Western Australia (Wagner & Pooley, 1986), among people residing in areas of tremolite contamination in Cyprus (McConnochie *et al.*, 1987) and New Caledonia (Luce *et al.*, 2000), and with non-occupational exposures in Europe (Magnani *et al.*, 2000), Italy (Magnani *et al.*, 2001), and California (Pan *et al.*, 2005).

Mesothelioma has also been reported to occur among household members of families of asbestos workers (Anderson *et al.*, 1976; Ferrante *et al.*, 2007).

2.3.5 Non-commercial asbestiform fibres

Several studies have described adverse health associations with the amphibole fibres that contaminated vermiculite mined in Libby, Montana, USA. These fibres were originally characterized as from the tremolite-actinolite series (IARC, 1987a); however, they were subsequently described by the US Geological Society as being composed of approximately 84% winchite, 11% richterite, and 6% tremolite (Meeker *et al.*, 2003). Sullivan (2007) reported SMRs, using cause of death data and expected mortality for the underlying cause of death based on national age-, race-, and sex-specific rates. Using a 15-year exposure lag, there were increased SMRs, mesothelioma defined by ICD-10 for deaths after 1999 (SMR, 14.1; 95%CI: 1.8–54.4; n = 2) and pleural cancer (SMR, 23.3; 95%CI: 6.3–59.5; n = 4). The only exposure–response modelling of mesothelioma was presented in the paper by McDonald *et al.*, based on 12 mesothelioma cases (McDonald *et al.*, 2004). Using Poisson regression, the mesothelioma mortality rate across increasing categories of exposure was compared with the rate in the lowest exposure category. For the cumulative exposure metric, the relative risk estimates were 1.0 (referent), 3.72, 3.42, and 3.68, based on 1, 4, 3, and 4, cases, respectively. The mean exposure level in these four quartiles was 8.6, 16.7, 53.2, and 393.8 f/mL–yr, respectively. It should be noted that the referent group was also at excess risk of dying from mesothelioma, i.e. there were 1–3 cases of mesothelioma observed in the referent group, which may have attenuated the observed effects.

A high incidence of mesothelioma was reported among residents of Biancavilla, Italy, a city in eastern Sicily (SMR, 7.21; 95%CI: 3.59–13.00). Reviewing of the work histories of the cases did not indicate an occupational explanation for these exposures, and thus environmental explanations for the mesothelioma excess were sought. Environmental studies have indicated that these mesotheliomas are most likely due to exposures to fluoro-edenite which is a newly recognized fibre that is very similar in morphology and composition to the tremolite-actinolite series (Comba *et al.*, 2003; Bruno *et al.*, 2006; Putzu *et al.*, 2006).

2.4 Other cancer sites

Beyond lung cancer and mesothelioma, the body of literature examining associations between asbestos and other cancers is more sparse. This reflects the fact that lung cancer and mesothelioma have been the principal areas of research

until relatively recently, and other cancers were often not considered in detail in published reports. Clinical and epidemiological studies that span the past five decades suggest, however, that asbestos may be associated with other cancers in addition to lung cancer and mesothelioma. To examine these associations in detail, the US IOM (2006) published a report evaluating the evidence relevant to causation of cancer of the pharynx, larynx, oesophagus, stomach, colon and rectum by asbestos. The present analysis draws on the IOM analysis and presents the most significant positive and negative studies for each anatomical site, with an emphasis on studies that presented data on dose–response as well as on published meta-analyses. Additionally, the present analysis examines the association between asbestos exposure and ovarian cancer, an association that was not examined by the IOM.

2.4.1 Cancer of the pharynx

See Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.5.pdf.

(a) Cohort Studies

The Working Group examined 16 cohort studies of asbestos and cancer of the pharynx. Some of these studies examined all cancers of the lips, oral cavity, and pharynx. Others restricted their examination to the pharynx itself. Two studies examined only cancers of the hypopharynx. The main findings are summarized in the following paragraphs.

Selikoff & Seidman (1991) observed an SMR for cancer of the oropharynx of 2.18 (95%CI: 1.62–2.91) among a cohort of 17800 male asbestos insulation workers across the USA and Canada. This is the cohort study with the largest number of deaths from pharyngeal cancer, a total of 48 deaths.

Piolatto *et al.* (1990) observed an SMR for cancer of the oropharynx of 2.31 (95%CI: 0.85–5.02; based on six deaths) in a cohort of 1058 asbestos miners in northern Italy exposed to chrysotile asbestos. No association was seen in this cohort between duration of occupational exposure to asbestos and risk of cancer of the pharynx.

Reid *et al.* (2004) observed an SMR for cancer of the pharynx of 1.88 (95%CI: 1.15–3.07; based on 16 deaths) in a cohort of 5685 crocidolite asbestos miners and millers in Western Australia.

Sluis-Cremer *et al.* (1992) observed an SMR for cancer of the lip, oral cavity and pharynx of 2.14 (95%CI: 1.03–3.94; based on 10 deaths) in a cohort of 7317 male asbestos miners in South Africa, some exposed to crocidolite and others to amosite. Cancer of the pharynx was defined in this population as cancer of the lip, oral cavity or pharynx. There was no excess mortality for cancer of the pharynx in the subcohort of amosite asbestos miners (SMR, 0.42; 95%CI: 0.00–1.97), but in the subcohort of crocidolite asbestos miners, the SMR for cancer of the pharynx was 2.94 (95%CI: 1.16–6.18).

Pira *et al.* (2005) observed an SMR for cancer fo the pharynx of 2.26 (95%CI: 0.90–4.65; based on seven deaths) in a cohort of 1996 workers in the asbestos textiles industry in Italy.

Other cohort studies of populations occupationally exposed to asbestos in a range of industries contained only small numbers of deaths from cancer of the pharynx (most < 10 deaths), were generally non-positive in their findings, and reported little evidence for dose–response relationships.

(b) Case–control studies

Case–control studies examining the association between asbestos exposure and cancer of the pharynx have two advantages over cohort studies:

1. they are able to collect more cases of this relatively uncommon malignancy; and
2. they are able to adjust for alcohol and tobacco consumption, the two most common causes

of cancer of the pharynx in developed and developing countries.

The present review included six case–control studies. Four of them adjusted for alcohol and tobacco consumption. The main findings are summarized in the following paragraphs.

Marchand *et al.* (2000) carried out a hospital-based, case–control study of 206 cases of cancer of the hypopharynx and 305 controls in France, and found a relative risk of 1.80 (95%CI: 1.08–2.99) in the 161 of their cases ever exposed to asbestos, adjusted for exposure to tobacco and alcohol.

Berrino *et al.* (2003) conducted a multicentre, case–control study of cancer of the hypopharynx in Europe, and found an odds ratio (OR) for "probable" exposure to asbestos of 1.8 (95%CI: 0.6–5.0). This study was restricted to analyses of cancers of the hypopharynx. For cases with "possible" exposure to asbestos, the odds ratio was 1.80 (95%CI: 0.90–3.90). These odds ratios were adjusted for exposure to tobacco and alcohol.

Zheng *et al.* (1992) conducted a population-based, case–control study of cancer of the pharynx in Shanghai, the People's Republic of China, with 204 incident cancer cases and 414 controls. The relative risk for asbestos exposure was 1.81 (95%CI: 0.91–3.60). Cigarette smoking and alcohol consumption were observed to be positively associated with cancer fo the pharynx. By contrast, increasing intake of certain fruits and vegetables, notably oranges, tangerines and Chinese white radishes, appeared to be associated with a reduced risk for cancer of the pharynx.

(c) Meta-analyses

The IOM (2006) conducted a meta-analysis of the published cohort studies examining the association between asbestos exposure and cancer of the pharynx. The IOM noted that the findings of the cohort studies were consistently positive. They calculated that the "estimated aggregated relative risk of cancer of the pharynx from any exposure to asbestos was 1.44 (95%CI: 1.04–2.00). "The IOM noted that few studies had evaluated dose–response trends, and that there was no indication of higher risks associated with more extreme exposures."

The IOM also conducted a meta-analysis of the case–control studies examining the association between asbestos exposure and cancer of the pharynx. The IOM reported the summary relative risk for cancer of the pharynx in people with "any" exposure to asbestos compared to people with no exposure to be 1.5 (95%CI: 1.1–1.7). The IOM observed that the studies were inconsistent, and that there was little evidence for a dose–response relationship.

2.4.2 Cancer of the larynx

See Table 2.5 online.

Cancer of the larynx in relation to asbestos exposure has been studied in a large number of cohort and case–control studies undertaken among occupationally exposed populations in North and South America, Europe, and Asia. (IOM, 2006).

(a) Cohort studies

Cohort studies of workers exposed occupationally to asbestos have found evidence for an association between asbestos exposure and cancer of the larynx across a broad range of industries. The Working Group reviewed 29 cohort studies encompassing 35 populations exposed to asbestos. Noteworthy findings from among these studies are summarized in the following paragraphs.

Selikoff & Seidman (1991) found an SMR for cancer of the larynx of 1.70 (95%CI: 1.01–1.69) among 17800 male insulation workers in the USA and Canada.

Musk *et al.* (2008) found an SMR for cancer of the larynx of 1.56 (95%CI: 0.83–2.67) among 6943 asbestos miners and millers from Western Australia, exposed predominantly to crocidolite

asbestos, when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring all subjects at the date last know to be alive, the SMR was 2.57 (95%CI: 1.37–4.39).

Reid *et al.* (2004) carried out a study of cancer incidence in this same Australian cohort, and found a significant increase in incidence of cancer of the larynx (SIR, 1.82; 95%CI: 1.16–2.85).

Piolatto *et al.* (1990) found an SMR for cancer of the larynx of 2.67 (95%CI: 1.15–5.25; based on eight deaths) in a cohort study of 1058 male asbestos miners in northern Italy. In the subset of this cohort with > 20 years' exposure to asbestos, the SMR for cancer of the larynx was 4.55 (95%CI: 1.47–10.61). There was evidence of a positive dose–response relationship between cumulative exposure to asbestos dust, measured as fibre–years, and risk of death from cancer of the larynx. The SMRs for cancer of the larynx were 1.43 (95%CI: 0.04–7.96) in workers with exposure < 100 fibre–years; 2.22 (95%CI: 0.27–8.02) in workers with exposure of 100–400 fibre–years; and 3.85 (95%CI: 1.25–8.98) in workers with cumulative exposure > 400 fibre–years.

Peto *et al.* (1985) found an overall SMR for cancer of the larynx of 1.55 (95%CI: 0.42–3.97; based on four deaths) in a cohort of 3211 asbestos-textile workers in the United Kingdom. When workers were subdivided according to time since first employment, and by duration of employment in "scheduled" (asbestos-exposed) areas of the plant, four deaths from cancer of the larynx were observed in the most heavily exposed group versus 1.53 expected (SMR, 2.55).

Pira *et al.* (2005) found an overall SMR for cancer of the larynx of 2.38 (95%CI: 0.95–4.90; based on seven deaths–all of them in male workers) in a cohort of 889 men and 1077 women employed in an asbestos textiles plant in Italy.

Raffn *et al.* (1989) found an overall SIR for cancer of the larynx of 1.66 (95%CI: 0.91–2.78) in a cohort study of 7986 men and 584 women employed in the asbestos-cement industry in Denmark However, in the subset with > 5 years employment, the SIR was 2.27 (95%CI: 0.83–4.95), and in the group first employed from 1928–40, the SIR was 5.50 (95%CI: 1.77–12.82).

(b) Case–control studies

Case–control studies are important in examining relationships between asbestos exposure and cancer of the larynx, because they overcome the relative rarity of the diagnosis in cohort studies, and also because they permit consideration of potential confounding by exposure to tobacco and alcohol, the two most important risk-factors for this malignancy in developed and developing countries.

The Working Group analysed 15 case–control studies of asbestos and cancer of the larynx. This analysis revealed that 14 of the 15 published studies had found evidence for a significantly positive association between asbestos exposure and cancer of the larynx; only one study (Luce *et al.*, 2000) reported an odds ratio below 1.0.

(c) Meta-analyses

The IOM conducted a meta-analysis of cohort studies examining the association between asbestos exposure and cancer of the larynx. For studies examining "any" versus no exposure, the summary relative risk was 1.4 (95%CI: 1.19–1.64). For studies comparing "high" exposure versus no exposure, the lower bound summary relative risk was 2.02 (95%CI: 1.64–2.47), and the upper bound summary relative risk was 2.57 (95%CI: 1.47–4.49).

The IOM also conducted a meta-analysis of the published case–control studies examining the association between asbestos exposure and cancer of the larynx (IOM, 2006). This meta-analysis calculated a summary relative risk of 1.43 (95%CI: 1.15–1.78), before adjusting for consumption of tobacco and alcohol. After adjusting for tobacco and alcohol consumption, the association of cancer of the larynx with

asbestos exposure persisted, with an adjusted summary relative risk of 1.18 (95%CI: 1.01–1.37).

2.4.3 Cancer of the oesophagus

See Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.6.pdf.

(a) Cohort studies

The Working Group examined 25 studies of cohorts occupationally exposed to asbestos. Notable findings from among these studies are:

Selikoff & Seidman (1991) found an SMR for cancer of the oesophagus of 1.61 (95%CI: 1.13–2.40) among a cohort of 17800 asbestos insulations workers across the USA and Canada. Selikoff & Seidman (1991) observed that cancer in asbestos workers is "very much related to latency," with most of the increased risk occurring only 25 or more years from the onset of occupational exposure to asbestos.

In a cohort of 10939 male and 440 female asbestos miners and millers in Quebec, Canada, exposed predominantly to chrysotile asbestos, followed through 1975, McDonald *et al.* (1980) reported that mortality for cancer of the oesophagus and stomach (the two were combined) was elevated (SMR, 1.27). Further follow-up through 1988 of a subset of this cohort, consisting of 5335 men, examined esophageal cancer mortality separate from stomach cance,r and found no excess mortality (SMR, 0.73; 95%CI: 0.35 – 1.34) (McDonald *et al.*, 1993).

Musk *et al.* (2008) found an SMR for cancer of the oesophagus was 1.01 (95%CI: 0.71–1.40) in a cohort study of 6943 asbestos miners from Western Australia followed through 2000, exposed predominantly to crocidolite asbestos, when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring all subjects at the date last known to be alive, the SMR was 1.20 (95%CI: 0.62–2.10).

Hein *et al.* (2007) found an SMR for cancer of the oesophagus of 1.87 (95%CI: 1.09–2.99) in a cohort of 3072 asbestos textile workers in South Carolina, occupationally exposed to chrysotile asbestos and followed through 2001.

Peto *et al.* (1985) found 11 deaths from cancer of the oesophagus versus 6.59 expected (SMR = 1.67; 95%CI: 0.83–2.99) in a cohort of 3211 male asbestos textile workers in the United Kingdom. For the subset of workers with 10+ years employment in "scheduled" (asbestos-exposed) areas of the plant and with 20+ years since first employment, the SMR for cancer of the oesophagus was 2.36 (95%CI: 0.49–6.91). For all workers in this cohort with < 20 years since first employment, two deaths for cancer of the oesophagus was observed versus 2.18 expected, and for workers with 20+ years since first employment, there were nine deaths from cancer of the oesophagus versus 4.4 expected (see Table 6 in Peto *et al.*, 1985).

Berry *et al.* (2000) found a 2-fold excess mortality for cancer of the oesophagus (SMR, 2.08; 95%CI: 1.07–3.63) among a cohort of over 5000 asbestos-exposed factory workers in the east end of London, United Kingdom, who had produced asbestos insulation boards, and who were followed for 30+ years. In the subset of workers within this population with "severe" asbestos exposure of more than 2 years' duration, the SMR for cancer of the oesophagus was 5.62 (95%CI: 1.82 – 13.11). And in the subset of women with "severe" exposure to asbestos of > 2 years, the SMR for cancer of the oesophagus was 9.09 (95%CI: 1.10–32.82).

Other cohort studies of various groups occupationally exposed to asbestos – asbestos-cement workers, friction products workers, and "generic" asbestos workers – yield generally non-positive results for cancer of the oesophagus.

(b) Case–control studies

The Working Group examined five case–control studies that examined the association between asbestos exposure and cancer of the oesophagus.

A case–control study in Quebec, Canada found an OR of 2.0 (95%CI: 1.1–3.8) for any exposure to asbestos among 17 patients diagnosed with squamous cell carcinoma of the oesophagus. (Parent *et al.*, 2000).

A case–control study conducted within a cohort of nearly 400000 Swedish construction workers found evidence for a positive association between asbestos exposure and adenocarcinoma of the oesophagus. Relative risk increased from 1.0 (reference) among workers with no asbestos exposure, to 1.7 (95%CI: 0.5–5.4) among those with "moderate" exposure, and to 4.5 (95%CI: 1.4–14.3) among those workers with "high" asbestos exposure, thus suggesting a positive dose–response relationship (Jansson *et al.*, 2005).

(c) Meta-analyses

Meta-analyses have been undertaken of the association between asbestos exposure and cancer of the oesophagus:

A meta-analysis by Frumkin & Berlin (1988) stratified studies according to SMR for lung cancer and also according to the percentage of deaths due to mesothelioma. The rationale is that a higher death rate for either lung cancer or mesothelioma is taken to be a surrogate index of higher cumulative exposure to asbestos. However, no association was observed between death rate for cancer of the oesophagus in the published cohorts by either lung cancer SMR or percentage of death for mesothelioma.

Meta-analyses by Edelman (1988) and by Goodman *et al.* (1999) did not detect an association between asbestos exposure and cancer of the oesophagus.

A meta-analysis by Morgan *et al.* (1985) that examined earlier studies, which tended to have heavier exposure, found a summary SMR for cancer of the oesophagus in asbestos-exposed workers of 2.14 (95%CI: 1.326–3.276). When cases of cancer of the oesophagus based on "best evidence" (pathological review) were deleted from these cohorts, the SMR remained elevated at 2.38 (95%CI: 1.45–3.68).

The IOM (2006) conducted a meta analysis of 25 cohort studies and reported a summary relative risk of 0.99 (95%CI: 0.78–1.27) for any exposure to asbestos versus no exposure. The IOM also examined the relative risk of "high" versus no exposure, and calculated a lower bound summary relative risk of 1.35 (95%CI: 0.81–2.27), and a higher bound summary relative risk of 1.43 (95%CI: 0.79–2.58). The IOM determined that there were too few case–control studies to permit a meta-analysis.

2.4.4 Cancer of the stomach

The Working Group reviewed 42 cohort studies and five population-based case–control studies that examined the association between asbestos and cancer of the stomach (See Table 2.6 online).

(a) Cohort studies

Notable findings among the cohort studies are:

Selikoff *et al.* (1964) reported a nearly 3-fold excess mortality for cancer of the stomach (12 observed versus 4.3 expected) in a population of 632 insulation workers in New York and New Jersey occupationally exposed to asbestos dust. Further analysis within this cohort (Selikoff *et al.*, 1979) found evidence of a dose–response relationship between duration of exposure to asbestos (in years), and risk of death from cancer of the stomach. The SMR for cancer of the stomach increased from 0.00 in workers exposed for < 20 years, to 4.00 (95%CI: 1.47 – 8.71) in those exposed for 20 –35 years, and to 3.42 (95%CI: 1.82 – 5.85) in those exposed for > 35 years.

Selikoff *et al.* (1967) found a modest, non-significant increase in risk of death for cancer of the stomach: 34 observed v. 29.4 expected, (SMR = 1.16;95%CI: 0.92 – 1.78) in a larger cohort study of 17800 insulation workers across the USA and Canada. No data on dose–response for cancer of the stomach were presented in this analysis.

Liddell *et al.* (1997) reported an overall SMR for cancer of the stomach of 1.24 (95%CI: 1.07 –1.48) in a study of 10918 asbestos miners and millers exposed predominantly to chrysotile asbestos, in Quebec, Canada. Within this cohort, a positive dose–response relationship was observed between cumulative exposure to asbestos dust (mcpf-year) and mortality for cancer of the stomach. Thus, for workers with cumulative dust exposure < 300, the SMR was 1.16; for workers with cumulative exposure of 300 – 400, the SMR was 1.29; for workers with cumulative exposure of 400 – 1000, the SMR was 1.21; and for workers in the highest exposure category, with cumulative exposure > 1000, the SMR was 3.21 (95%CI: 1.87 –5.14). An additional finding in this cohort was a modest interaction between cumulative asbestos exposure, cigarette smoking, and mortality from cancer of the stomach.

Musk *et al.* (2008) found an SMR for cancer of the stomach of 1.01 (95%CI: 0.71 – 1.40) in a cohort of 6943 asbestos miners and millers exposed predominantly to crocidolite asbestos in Wittenoom, Western Australia, followed through the end of 2000, and when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring subjects at the date last known to be alive, the SMR was 1.71 (95%CI: 1.20–2.35).

Reid *et al.* (2004) conducted a nested case–control study within this same Australian cohort, and found a positive exposure-response relationship between cancer of the stomach and cumulative exposure to asbestos (test for trend, $P = 0.057$). No association was seen between cancer of the stomach and either time since first exposure or year of starting work with asbestos. Smoking status was associated with cancer of the stomach, but not significantly.

Meurman *et al.* (1974) found a non-significant increase in SMR for cancer of the stomach: SMR = 1.42 (95%CI: 0.76 – 2.43) in a cohort of 736 asbestos miners in Finland exposed to anthophyllite asbestos.

Berry *et al.* (2000) found a modest, non-significant increased risk for death from cancer of the stomach: 28 observed versus 23.1 expected (SMR, 1.21; 95%CI: 0.81–1.75) in a British study of factory workers producing asbestos insulation in the east end of London.

Strongly positive dose–response associations between cumulative asbestos response and cancer of the stomach were observed in two cohort studies of Chinese factory workers – one in Beijing and the other in Qingdao; relative risks for cancer of the stomach were 4.4 and 2.4, respectively (Zhu & Wang, 1993; Pang *et al.*, 1997).

Raffn *et al.* (1989) observed 43 deaths from cancer of the stomach versus 30.09 expected (SMR, 1.43; 95%CI: 1.03 – 1.93) in a cohort of 7986 men employed from 1928–84 in the asbestos cement industry in Denmark.

Enterline *et al.* (1987) observed a SMR for cancer of the stomach of 1.80 (95%CI: 1.10–2.78) in a cohort of 1074 retired US asbestos workers.

Epidemiological studies of cohorts with asbestos-related diseases – asbestosis and benign pleural disease – have not found increased mortality for cancer of the stomach (Germani *et al.*, 1999; Karjalainen *et al.*, 1999; Szeszenia-Dabrowska *et al.*, 2002).

(b) Case–control studies

Case–control studies exploring the relationship between asbestos exposure and cancer of the stomach yield inconsistent results. The Working Group reviewed five case–control studies. Notable findings are these:

A study from Poland (Krstev *et al.*, 2005) found an OR for cancer of the stomach of 1.5 (95%CI: 0.9–2.4) for workers ever exposed to asbestos, and of 1.2 (95%CI: 0.6–2.3) for workers with 10 or more years of exposure to asbestos.

The largest case–control study to examine the association between asbestos and cancer of the stomach (Cocco *et al.*, 1994) reported an odds ratio of 0.7 (95%CI: 0.5–1.1) for workers ever exposed to asbestos, and of 1.4 (95%CI: 0.6–3.0) for those with 21+ years of exposure to asbestos.

The most strongly positive case–control study linking asbestos to cancer of the stomach is the case–control study, cited above, nested within the Western Australia mining cohort (Reid *et al.*, 2004).

(c) Meta-analyses

Several meta-analyses have been undertaken of the association between asbestos exposure and cancer of the stomach.

A meta-analysis by Frumkin & Berlin (1988) stratified studies according to SMR for lung cancer and also according to percentage of deaths due to mesothelioma. Frumkin & Berlin found in cohorts where the SMR for lung cancer was < 2.00 that the SMR for cancer of the stomach was 0.91 (95%CI: 0.71–1.16). By contrast, when the SMR for lung cancer was > 2.00, the SMR for cancer of the stomach increased to 1.34 (95%CI: 1.07–1.67).

Gamble (2008) reported that point estimates for cancer of the stomach mortality tended towards 1.0 when the excess risk for lung cancer were less than 4-fold, but "tended to be somewhat elevated when lung cancer relative risks were 4-fold or greater." Gamble observed further that "combined relative risks for cancer of the stomach stratified by lung cancer categories showed a suggestive trend, with a significant deficit (0.80) when lung cancer SMRs were <1.0 that increased monotonically to a significant 1.43-fold excess in the studies with lung cancer SMRs > 3.0." Gamble observed no trend for increasing SMR for cancer of the stomach with increasing percentage of deaths from mesothelioma (Gamble, 2008).

The IOM (2006) conducted a meta-analysis of 42 cohort studies examining the association between asbestos exposure and cancer of the stomach. The IOM noted that the "majority of cohort relative risk estimates for cancer of the stomach exceed the null value (1.0), indicating excesses, although estimates varied considerably in strength." In cohorts that compared "any" versus no exposure, the summary relative risk was 1.17 (95%CI: 1.07–1.28). The IOM notes that with respect to dose–response, the summary estimates were stable. Thus in the cohorts that compared "high" versus no exposure, the lower bound summary relative risk was 1.31 (95%CI: 0.97–1.76), and the higher bound summary relative risk, 1.33 (95%CI: 0.98–1.79).

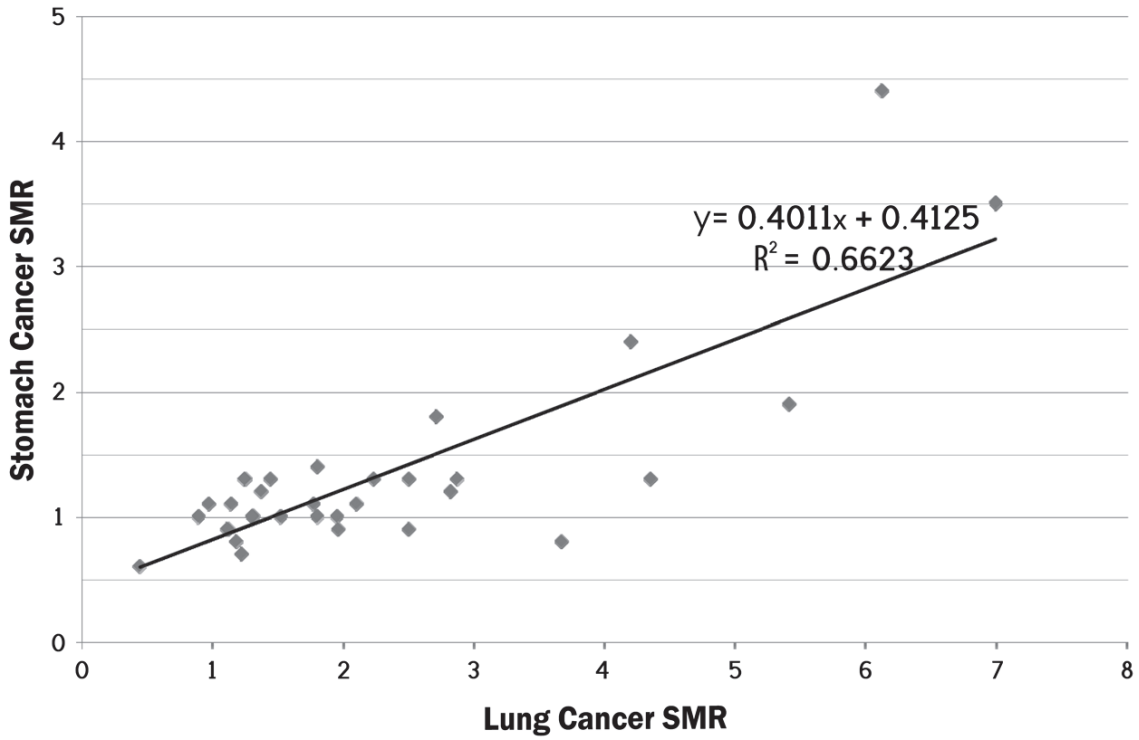
The IOM conducted a meta-analysis of the five case–control studies resulting in a combined relative risk of 1.11 (95%CI: 0.76–1.64). The summary odds ratio increased when only extreme exposure was considered (OR, 1.42; 95%CI: 0.92–2.20)

The Working Group developed a scatter plot comparing SMRs for lung cancer with SMRs for cancer of the stomach in the same cohorts. A positive trend was observed between the two, and the correlation coefficient (r2) = 0.66, see Fig. 2.1.

(i) Asbestos in drinking-water and cancer of the stomach

Ecological correlational studies conducted from the 1960s into the early 1980s suggested an association between asbestos in drinking-water and cancer of the stomach. These studies correlated population exposure to asbestos in water supplies with population cancer rates. Levy *et al.* (1976) reported an excess in cancer of the stomach among persons in Duluth, MN, USA exposed to taconite asbestos in drinking-water. Wigle (1977) saw an excess of male cancer of the stomach among some exposed to asbestos in drinking-water in Quebec. Conforti *et al.* (1981)

Fig 2.1 Stomach & lung cancer correlation in asbestos cohorts



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saw a similar association in the San Francisco Bay area, USA. Polissar *et al.* (1982) examined cancer incidence and mortality among residents of the Puget Sound area, USA, in relation to asbestos in regional drinking-water. They observed no association between asbestos exposure and cancer of the stomach. A similarly negative study was observed in a study conducted in Woodstock, NY, USA (Howe *et al.*, 1989).

Kjærheim *et al.* (2005) examined cancer of the stomach incidence in Norwegian light-house keepers exposed to asbestos in drinking-water. They found an SIR for cancer of the stomach in the entire cohort of 1.6 (95%CI: 1.0–2.3). In the subcohort with "definite" exposure to asbestos, the SIR was 2.5 (95%CI: 0.9–5.5). In those members of the definite exposure subcohort followed for 20+ years, the SIR was 1.7 (95%CI: 1.1–2.7).

Cantor (1997) conducted a systematic review of the epidemiological literature on exposure to asbestos in drinking-water and cancer of the stomach, and concluded that the available data were inadequate to evaluate the cancer risk of asbestos in drinking-water.

Marsh (1983) conducted a critical analysis of 13 epidemiological studies of asbestos and drinking-water conducted in the USA and Canada, and found no consistent pattern of association.

2.4.5 Cancer of the colorectum

The Working Group examined data from 41 occupational cohorts and 13 case–control studies that reported data on associations between asbestos exposure and cancer of the colon and rectum (See Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.7.pdf). The Working Group made the decision to combine information on these two sites, although a few comments in several places in the text about the two sites considered separately have also been made.

(a) Cohort studies

An association between occupational exposure to asbestos and cancer of the colorectum was first reported in 1964 by Selikoff *et al.* in a cohort of 632 male insulation workers in New York and New Jersey, USA (Selikoff *et al.*, 1964). Further analysis of this cohort found a positive relationship between duration of work with asbestos and risk of cancer of the colorectum, in that the SMR increased from 0.00 (95%CI: 0.00–18.45) in workers with < 20 years exposure, to 3.68 (95%CI: 1.48–7.59) among workers with 20–35 years' exposure, and to 2.58 (95%CI: 1.48–4.19) among workers with the longest duration of exposure, > 35 years (Selikoff & Hammond, 1979).

Selikoff *et al.* (1967), in a second report, found an association between occupational exposure to asbestos and cancer of the colorectum in a population of 17800 asbestos insulators across the USA and Canada (SMR, 1.37; 95%CI: 1.14–1.64).

Seidman *et al.* (1986) reported an elevated mortality from cancer of the colorectum in a population of 820 male factory workers in Paterson, NJ, USA, exposed to amosite asbestos (SMR, 2.77; 95%CI: 1.16–2.80). They noted that cancer of the colorectum in asbestos workers tended to be a disease of long latency; they reported that the ratio of observed to expected deaths increased with increasing interval since initial exposure to asbestos.

McDonald *et al.* (1980) reported an overall SMR for cancer of the colorectum of only 0.78 in a study of 10939 men and 440 women workers employed as asbestos miners and millers in Quebec with predominant exposure to chrysotile asbestos. Additionally, however, McDonald *et al.* reported a "clear trend for SMRs to be higher, the heavier the exposure." Thus with increasing levels of cumulative occupational exposure to asbestos dust, relative risks for cancer of the colorectum increased in this cohort from 1.00 in workers with less than 30 mpcf–y cumulative exposure, to 0.93 in workers with 30–300 mpcf–y, to 1.96 in workers with 300–1000 mpcf–y, and then in the group with heaviest exposure, > 1000 mpcf–y, to 5.26.

Albin *et al.* (1990) found an overall SMR for cancer of the colorectum of only 1.5 (95%CI: 0.7–3.0) in a cohort of 1465 asbestos-cement workers in Sweden. A positive association between asbestos exposure and cancer of the colorectum was reported, but when cancer of the colorectum mortality was examined by individual cumulative exposure to asbestos, measured as fibre–years/mL, the SMR was 1.3 (95%CI: 0.5–2.9) for those workers with cumulative exposure of < 15 fibre–years/mL; for those with cumulative exposure of 15–39 fibre–years/mL, the SMR was 1.1(95%CI: 0.3–3.9); and for those workers in highest exposure category with > 40 fibre–years/mL, the SMR for cancer of the colorectum was 3.4 (95%CI: 1.2–9.5). Diagnosis in all but one of the cancers in the highest exposure category was verified by pathological review, and no case of certified or probable mesothelioma was found. The trend towards increasing mortality from cancer of the colorectum with increasing cumulative exposure to asbestos was statistically significant ($P = 0.04$). A similar trend was seen for cancer of the colorectum morbidity.

Excess mortality from colon cancer was observed in a heavily exposed cohort of over

5000 workers in the east end of London, who had produced asbestos insulation board and were followed for 30+ years (Berry *et al.*, 2000). The overall SMR for colon cancer in this cohort was 1.83 (95%CI: 1.20–2.66). There was evidence for a positive dose–response relationship, in that excess mortality from colon cancer was confined to men who had worked as laggers or had been severely exposed for more than 2 years. This positive trend was statistically significant ($P = 0.017$).

In a cohort comprised of family members of men who had been employed in an asbestos-cement factory in Casale Monferrato, Italy, Ferrante *et al.* (2007) examined cancer mortality. Among women with domestic exposure to asbestos, 21 deaths from cancer of the "intestine and rectum" versus 16.0 expected (SMR, 1.31; 95%CI: 0.81–2.0) were observed. For cancer of the rectum, ten deaths versus five expected (SMR, 2.00; 95%CI: 0.96–3.69) were observed.

Several other cohort studies of occupationally exposed populations in a variety of industries have also found evidence for an association between asbestos exposure and cancer of the colorectum (Puntoni *et al.*, 1979: Hilt *et al.*, 1985; Jakobsson *et al.*, 1994; Raffn *et al.*, 1996; Szeszenia-Dabrowska *et al.*, 1998; Smailyte *et al.*, 2004).

Jakobsson *et al.* (1994) examined colon cancer by anatomical location in asbestos-cement workers, and observed an increased incidence of malignancy in the right side of the colon, but not in the left side.

A report on incidence of cancer of the colorectum from the Beta-Carotene and Retinol Efficacy Trial (CARET) found a relative risk of 1.36 (95%CI: 0.96–1.93) among 3987 heavy smoker participants occupationally exposed to asbestos as compared to smoker participants not exposed to asbestos (Aliyu *et al.*, 2005). Of note was the finding that the relative risk for cancer of the colorectum was 1.54 (95%CI: 0.99–2.40) among participants with asbestos-induced pleural plaques. The investigators interpreted the presence of pleural plaques as a marker for heavy individual exposure to asbestos. Risk for cancer of the colorectum also increased with worsening pulmonary asbestosis ($P = 0.03$ for trend). It was reported that a "dose–response trend based on years of asbestos exposure was less evident".

(b) *Case–control studies*

Evidence from case–control studies of asbestos and cancer of the colorectum is in general less strong than the evidence from the cohort studies. However, case–control studies from the Nordic countries and the USA have, however, reported significant increases in asbestos-associated odds ratios in occupationally exposed poulations (Fredriksson *et al.*, 1989; Gerhardsson de Verdier *et al.*, 1992; Vineis *et al.*, 1993; Kang *et al.*, 1997; Goldberg *et al.*, 2001).

Consideration of latency since first exposure appears to be an important factor in assessing these studies. Thus, Gerhardsson de Verdier *et al.* (1992) examined incidence of cancer of the colorectum by interval since first occupational exposure and observed "for subjects exposed to asbestos, the risks were highest when the latency period was more than 39 years." Gerhardsson de Verdier *et al.* observed further that the relative risk for cancer of the right colon was 2.6 (95%CI: 1.2–5.9) among workers exposed to asbestos, and that for malignancy of the left colon, only 0.5 (95%CI: 0.1–1.9).

Other cohort and case–control studies have not found evidence for an association between asbestos exposure and cancer of the colorectum (Gardner *et al.*, 1986; Hodgson & Jones, 1986; Garabrant *et al.*, 1992; Dement *et al.*, 1994; Demers *et al.*, 1994; Tulchinsky *et al.*, 1999; Hein *et al.*, 2007; Loomis *et al.*, 2009).

(c) *Meta-analyses*

Some of these meta-analyses have stratified studies according to the standardized mortality ratio for lung cancer or the percentage of deaths due to mesothelioma:

Morgan *et al.* (1985) found a summary standardized mortality ratio for cancer of the colorectum of 1.13 (95%CI: 0.97–1.30). This was reduced to 1.03 (95%CI: 0.88–1.21) after deleting cases in which the diagnosis of cancer of the colorectum was based on "best evidence" (pathological review) rather than death certificate data.

Frumkin & Berlin (1988) found in cohorts where the standardized mortality ratio for lung cancer was < 2.00 that the standardized mortality ratio for cancer of the colorectum was 0.86 (95%CI: 0.69–1.09). By contrast, when the standardized mortality ratio for lung cancer was > 2.00, the standardized mortality ratio for cancer of the colorectum increased to 1.61 (95%CI: 1.34–1.93).

Homa *et al.* (1994) found an elevated summary standardized mortality ratio for cancer of the colorectum in cohorts exposed to serpentine asbestos that had an standardized mortality ratio for lung cancer > 2.00 (summary standardized mortality ratio for cancer of the colorectum, 1.73; 95%CI: 0.83–3.63), and also in cohorts exposed to a mix of amphibole and serpentine asbestos that had a standardized mortality ratio for lung cancer > 2.00 (summary standardized mortality ratio for cancer of the colorectum, 1.48; 95%CI: 1.24–1.78). Among cohorts exposed to amphibole asbestos, the standardized mortality ratio for cancer of the colorectum was elevated regardless of the standardized mortality ratio for lung cancer. Homa *et al.* (1994) saw similar trends between standardized mortality ratio for cancer of the colorectum and percentage of deaths from mesothelioma.

Gamble (2008) reported that there was "tendency for CRC [cancer of the colorectum] risk ratios to be elevated when lung cancer risk ratios are >4" and further noted a significantly elevated standardized mortality ratio of 1.60 (95%CI: 1.29–2.00) for cancer of the colorectum when the standardized mortality ratio for lung cancer exceeds 3.00. Gamble (2008) observed no trend in cancer of the colorectum mortality with increasing percentage of deaths due to mesothelioma. Gamble saw no association between asbestos exposure and rectal cancer.

The IOM (2006) conducted a meta-analysis of cohort studies examining the association between asbestos exposure and cancer of the colorectum. In studies that compared "any" versus no exposure, the summary relative risk was 1.15 (95%CI: 1.01–1.31). For studies comparing "high" versus no exposure, the lower-bound summary relative risk was 1.24 (95%CI: 0.91–1.69), and the upper-bound summary relative risk, 1.38 (95%CI: 1.14–1.67).

The IOM also conducted a meta-analysis of the published case–control studies. Overall, 13 studies comparing "any" versus no exposure yielded a summary relative risk of 1.16 (95%CI: 0.90–1.49).

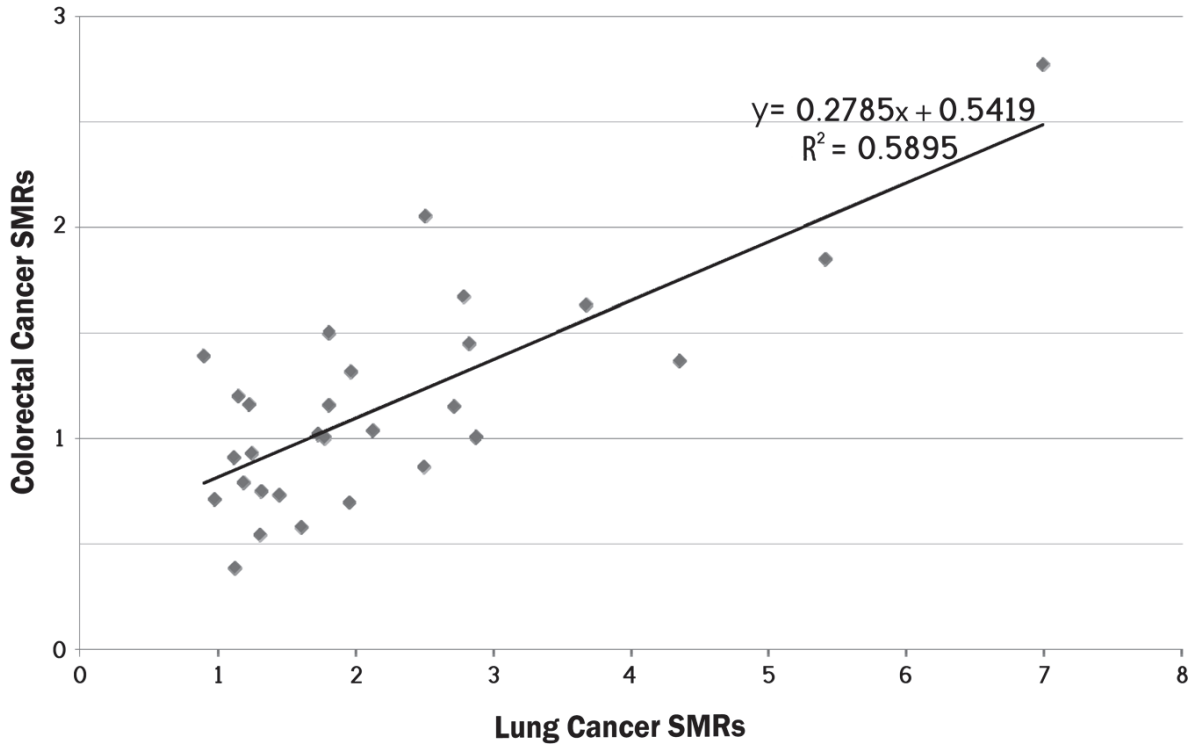
The *IARC Monograph* 100C Working Group developed a scatter plot comparing standardized mortality ratios for lung cancer with standardized mortality ratios for cancer of the colorectum in the same cohorts. The trend was positive with a correlation coefficient (r2) of 0.59, see Fig. 2.2.

(i) Asbestos in drinking-water and cancer of the colorectum

Ecological correlational studies conducted from the 1960s into the early 1980s suggested an association between asbestos in drinking-water and cancer of the colon. These studies correlated population exposure to asbestos in water supplies with population cancer rates. Polissar *et al.* (1982) examined cancer incidence and mortality among residents of the Puget Sound area, USA, in relation to asbestos in regional drinking-water. No association between asbestos exposure and colon cancer was observed. A similarly negative study was observed in a study conducted in Woodstock, NY, USA (Howe *et al.*, 1989).

Kjærheim *et al.* (2005) examined colon cancer incidence in Norwegian light-house keepers exposed to asbestos in drinking-water. The standardized incidence ratio for colon cancer in

Fig 2.2 Colorectal & lung cancer correlation in asbestos cohorts



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the entire cohort was 1.5 (95%CI: 0.9–2.2). In the subcohort with "definite" exposure to asbestos, the standardized incidence ratio was 0.8 (95%CI: 0.1–2.9). In those members of the definite exposure subcohort followed for 20+ years, the standardized incidence ratio was 1.6 (95%CI: 1.0–2.5).

Cantor (1997) conducted a systematic review of the epidemiological literature on exposure to asbestos in drinking-water and colon cancer and concluded that the data were inadequate to evaluate colon cancer risk of asbestos in drinking-water.

Marsh (1983) conducted a critical analysis of 13 epidemiological studies of asbestos and drinking-water conducted in the USA and Canada and found no consistent pattern of association.

2.4.6 Cancer of the ovary

The published literature examining the association between asbestos exposure and cancer of the ovaries is relatively sparse, because the workforce occupationally exposed to asbestos in such occupations as mining, milling shipyard work, construction and asbestos insulation work has been predominantly male. An examination of the association between asbestos and ovarian cancer was not undertaken by the IOM (2006).

See Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.8.pdf.

(a) Cohort studies

The Working Group examined 11 cohort studies that examined the association between asbestos exposure and ovarian cancer in 13 populations, ten with occupational exposure to asbestos and three with community-based or residential exposure.

Acheson *et al.* (1982) examined a cohort in the United Kingdom consisting of two groups of women in separate factories (n = 1327), employed in the manufacture of asbestos-containing gas masks before and during World War II. One factory had used crocidolite asbestos, and the other had used chrysotile. Among 757 women in the plant that used crocidolite, 12 deaths from ovarian cancer were observed versus. 4.4 expected (SMR, 2.75; 95%CI: 1.42–4.81). Among 570 women in the plant that used chrysotile asbestos, five deaths were observed for ovarian cancer versus 3.4 expected (SMR, 1.48; 95%CI: 0.48–3.44).

Wignall & Fox (1982) conducted a 30-year, follow-up mortality study of a population of 500 women in the United Kingdom employed in the manufacture of asbestos-containing gas masks before and during World War II. The type of asbestos used was crocidolite. A total of six deaths from ovarian cancer were obserevd versus. 2.8 expected (SMR, 2.13). When the cohort was subdivided according to degree of exposure to asbestos, the highest mortality from ovarian cancer was found among the subgroup definitely exposed to asbestos from the early 1940s (SMR, 14.81; $P < 0.01$). Overall five deaths from ovarian cancer were found among women definitely exposed to asbestos (versus 0.63 expected), whereas none were found among women definitely not exposed to asbestos (versus 0.40 expected).

To address potential misclassification of some deaths in this cohort recorded on death certificates as ovarian cancer as opposed to peritoneal mesothelioma, Wignall & Fox (1982) conducted a histopathological review of the cases cases of diagnosed ovarian cancer for which tissue material was available. One of these three cases was found to be peritoneal mesothelioma, while the diagnosis of ovarian cancer was sustained in the other two cases.

In a cohort study of 700 women factory workers employed in an asbestos-board insulation manufacturing company in the east end of London and followed for 30+ years, Berry *et al.* (2000) observed nine deaths from ovarian cancer versus 3.56 expected (SMR, 2.53; 95%CI: 1.16–4.80) (Berry *et al.*, 2000), with evidence for a positive exposure–response relationship. Among women with low-to-moderate exposure to asbestos, two deaths were observed versus 0.54 expected; in the subset with "severe" asbestos exposure of < 2 years' duration, two deaths were observed versus 2.12 expected. (SMR, 0.94); and among women with severe exposure of > 2 years' duration, five deaths from ovarian cancer were observed versus 0.90 expected (SMR, 5.35).

An assessment was performed of the significance of the positive exposure–response trend (P = 0.18). To address the potential misclassification of some deaths in this cohort having been recorded as ovarian cancer as opposed to peritoneal mesothelioma, Newhouse *et al.* (1972) conducted a histopathological review of the four deaths that by 1972 had been recorded as due to ovarian cancer; three of the four had occurred in women with severe and prolonged exposure to asbestos. Histological material was available for two of these cases. In both, the diagnosis of ovarian cancer was confirmed.

Reid *et al.* (2008) reported on cancer mortality in a cohort of 2552 women and girls who lived in the crocidolite asbestos mining town of Wittenoom in Western Australia during 1943–92, who were not involved in asbestos

mining and milling. Environmental contamination of the town with asbestos dust is reported to have been extensive. The women's exposure was environmental and not occupational. There were nine deaths from ovarian cancer in this cohort (SMR, 1.26; 95%CI: 0.58–2.40).

Reid *et al.* (2009) conducted a cancer incidence study in the same cohort of 2552 women and girls in Western Australia with environmental exposure to crocidolite asbestos. Additionally, they examined cancer incidence in 416 women who had worked in various capacities in the Wittenoom crocidolite asbestos mines and mills. Among community residents, ten incident cases of ovarian cancer were observed (SIR, 1.18; 95%CI: 0.45–1.91). Among women workers employed in the asbestos factory, one case of ovarian cancer was observed (SIR, 0.49; 95%CI: 0.01–2.74).

To address the possibility that some diagnosed cases of ovarian cancer in this cohort might in fact have been cases of peritoneal mesothelioma, Reid *et al.* (2009) examined pathological material from nine of their cases. The diagnosis of ovarian cancer was sustained in every case.

Pira *et al.* (2005) conducted a cohort study of 1077 women employed for at least one month during 1946–84 in an asbestos-textile factory in Italy, and followed up to 1996. A variety of types of asbestos were used in the factory, including crocidolite. A non-significantly increased standardized mortality ratio of 2.61 was observed for cancer of the ovary, based on five deaths. Among women in this cohort with ≥ 10 years of employment with asbestos, the standardized mortality ratio for ovarian cancer was 5.73, based on three deaths. Among women with ≥ 35 years since first employment, the standardized mortality ratio for ovarian cancer was 5.37, based on two deaths. This cohort was heavily exposed to asbestos, as supported by a standardized mortality ratio for lung cancer among women of 5.95, and by the occurrence of 19 deaths from mesothelioma (12%) among 168 total deaths in women.

Magnani *et al.* (2008) examined cancer mortality among a cohort of former workers at a now closed asbestos-cement factory in Casale Monferrato, Italy. A mix of crocidolite and chrysotile asbestos was used in this factory. Among women workers, there was an excess of ovarian cancers: nine observed versus 4.0 expected (SMR, 2.27; $P < 0.05$). Among women workers with 30 or more years exposure, the standardized mortality ratio for ovarian cancer was 2.97. Bertolotti *et al.* (2008) described the same findings in the same cohort [in Italian].

Ferrante *et al.* (2007) examined cancer mortality in a cohort consisting of family members of men who had been employed in the asbestos-cement factory in Casale Monferrato, Italy, described in the preceding paragraph. Exposure was to a mix of crocidolite and chrysotile. Among women with domestic exposure to asbestos, 11 deaths from ovarian cancer were observed versus 7.7 expected (SMR, 1.42; 95%CI: 0.71–2.54).

Germani *et al.* (1999) examined mortality from ovarian cancer in a cohort of 631 women workers in Italy who had been compensated for asbestosis. The type of fibre to which the women were exposed was not specified. In the total cohort, there were nine deaths from ovarian cancer (SMR, 4.77; 95%CI: 2.18–9.06). In the subset of women from the asbestos-textile industry, there were four deaths from ovarian cancer (SMR, 5.26; 95%CI: 1.43–13.47). In the subcohort from the asbestos cement industry, there were five deaths from ovarian cancer (SMR = 5.40; 95%CI: 1.75 – 12.61).

Rösler *et al.* (1994) examined cancer mortality in a cohort of 616 women workers in Germany who had been occupationally exposed to asbestos. Proportionate mortality was computed according to cause of death. A total of 95% of the asbestos used in Germany at this time was chrysotile, but the authors state that "admixture of crocidolite cannot be excluded, particularly in the manufacture of asbestos textile." Two deaths

from ovarian cancer were observed versus 1.8 expected (SMR, 1.09; 95%CI: 0.13–3.95).

(i) Population-based cohort studies

Vasama-Neuvonen *et al.* (1999) conducted a case–control study of ovarian cancer of occupational exposures in Finland. The asbestos fibre type was not specified and the standardized incidence ratio was 1.30 (95%CI: 0.9–1.80) between ovarian cancer and exposure to "high levels of asbestos."

Pukkala *et al.* (2009) examined the incidence of ovarian cancer among women employed in various occupational categories in Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden). Among the groups examined were plumbers, a group with known occupational exposure to asbestos. Fibre type was not specified. A total of four ovarian cancers were observed in these women plumbers. The standardized incidence ratio was 3.33 (95%CI: 0.91–8.52)

(b) Case–control studies

Langseth & Kjærheim (2004) conducted a nested case–control study to examine the association between asbestos exposure and ovarian cancer within a cohort of female pulp and paper workers in Norway that had previously been found to have excess mortality from ovarian cancer (37 ovarian cancers observed versus 24 expected; SIR, 1.50; 95%CI: 1.07–2.09). The asbestos fibre type was not specified. In the case–control study, the odds ratio for occupational exposure to asbestos, based on 46 cases of ovarian cancer, was 2.02 (95%CI: 0.72–5.66).

2.5 Synthesis

The Working Group noted that a causal association between exposure to asbestos and cancer of the larynx was clearly established, based on the fairly consistent findings of both the occupational cohort studies as well as the case-controlcase–control studies, plus the evidence for positive exposure–response relationships between cumulative asbestos exposure and laryngeal cancer-cancer of the larynx reported in several of the well-conducted cohort studies. This conclusion was further supported by the meta-analyses of 29 cohort studies encompassing 35 populations and of 15 case-controlcase–control studies of asbestos exposure and laryngeal cancercancer of the larynx undertaken by the IOM (2006). However, there is insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause laryngeal cancercancer of the larynx.

The Working Group noted that a causal association between exposure to asbestos and cancer of the ovary was clearly established, based on five strongly positive cohort mortality studies of women with heavy occupational exposure to asbestos (Acheson *et al.*, 1982; Wignall & Fox, 1982; Germani *et al.*, 1999; Berry *et al.*, 2000; Magnani *et al.*, 2008). The conclusion received additional support from studies showing that women and girls with environmental, but not occupational exposure to asbestos (Ferrante *et al.*, 2007; Reid *et al.*, 2008, 2009) had positive, though non-significant, increases in both ovarian cancer incidence and mortality.

The Working Group carefully considered the possibility that cases of peritoneal mesothelioma may have been misdiagnosed as ovarian cancer, and that these contributed to observed excesses. Contravening that possibility is the finding that three of the studies cited here specifically examined the possibility that there were misdiagnosed cases of peritoneal mesothelioma, and all failed to find sufficient numbers of misclassified cases. The Working Group noted that the possibility of diagnostic misclassification had probably diminished in recent years because of the development of new immunohistochemical diagnostic techniques.

The conclusion of the Working Group received modest support from the findings of

non-significant associations between asbestos exposure and ovarian cancer in two case–control studies (Vasama-Neuvonen *et al.*, 1999; Langseth & Kjærheim, 2004).

And lastly, the finding is consistent with laboratory studies documenting that asbestos can accumulate in the ovaries of women with household exposure to asbestos (Heller *et al.*, 1996) or with occupational exposure to asbestos (Langseth *et al.*, 2007).

The study by Heller *et al.* (1996) was a histopathological study of ovaries from 13 women who had household contact with men who had documented exposure to asbestos, and of 17 women who gave no history of potential for asbestos exposure. The study found "significant asbestos fibre burdens" in the ovaries of nine (60.2%) of the exposed women and in only six (35%) of the unexposed women. Three of the exposed women had asbestos fibre counts in ovarian tissue of over 1 million fibres per gram (wet weight). By contrast, only one of the 17 women without household exposure had counts in that range.

The study by Langseth *et al.* (2007) found approximately 3–4 × 105 asbestos fibres per gram (net weight) in normal ovarian tissue taken from 2/46 patients with ovarian adenocarcinoma. It is unclear how many of these fibres were verified as asbestos because it is stated in the publication that three chrysotile and one crocidolite asbestos fibres were identified in Case 1, and two anthophyllite and one chrysotile fibre were identified in Case 2. This small number of confirmed asbestos fibres in only two of the patients could be due to sample contamination. Technical caveats associated with quantification of asbestos fibre tissue burdens are discussed in Section 4 of this *Monograph* and in IOM (2006).

Further discussion of the biological plausibility of an association between asbestos exposure and ovarian cancer is to be found in Section 4 of this *Monograph*.

The Working Group noted a positive association between exposure to abestos and cancer of the pharynx, based on the fairly consistent positive findings in a series of well conducted cohort studies of populations occupationally exposed to asbestos (Selikoff & Seidman, 1991; Sluis-Cremer *et al.*, 1992; Reid *et al.*, 2004; Pira *et al.*, 2005) as well as on the positive findings of three case–control studies (Zheng *et al.*, 1992; Marchand *et al.*, 2000; Berrino *et al.*, 2003). This conclusion was further supported by the findings of the meta-analysis conducted by the IOM. While tobacco smoking and alcohol consumption are clearly the dominant risk factors for cancer of the pharynx in industrialized countries, these associations between cancer of the pharynx and asbestos remained evident in several studies when tobacco and alcohol exposures were considered. The Working Group observed that the strongest associations between asbestos exposure and cancer of the pharynx were seen in studies that specifically examined cancer of the hypopharynx, the portion of the pharynx that is located closest to the larynx. However, there is insufficient information in the published literature to discern whether there are any differences among asbestos fibre types in their ability to cause cancer of the pharynx.

The Working Group noted a positive association between exposure to abestos and cancer of the stomach, based on the positive associations between asbestos exposure and death from stomach cancer observed in several of the cohort studies with heaviest asbestos exposure (Selikoff *et al.*, 1964; Enterline *et al.*, 1987; Raffn *et al.*, 1989; Liddell *et al.*, 1997; Musk *et al.*, 2008). The conclusion was further supported by the positive dose–response relationships observed between cumulative asbestos exposure and stomach cancer mortality in several cohort studies (Selikoff & Hammond., 1979; Zhang & Wang, 1984; Liddell *et al.*, 1997; Pang *et al.*, 1997). It was supported by the results of two large and well performed meta-analyses (Frumkin & Berlin, 1988; Gamble, 2008). It received borderline support from the IOM meta-analysis of cohort

studies, and also from the IOM meta-analysis of case–control studies, which show an especially strong relationship when only extreme exposures are considered. It was supported by the comparison developed by the Working Group between standardized incidence ratios for lung cancer and stomach cancer.

Positive associations between asbestos exposure and stomach cancer and positive dose–response relationships are most likely to be seen in studies of populations with prolonged heavy exposure to asbestos that had long-term follow-up, and that incorporated high-quality assessments of exposure. The less detailed assessments of exposure found in many of the published studies would have tended to bias study results towards the null, and thus impede recognition of an association between asbestos exposure and stomach cancer, even if such an association were truly present.

[The Working Group noted that heavy occupational exposure to dust, as had likely occurred in the case of the Quebec asbestos cohort, could have been an effect modifier. Low socioeconomic status is also a potential confounder.]

However, there was insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause stomach cancer. In the study by Liddell *et al.* (1997) exposure was to virtually pure chrysotile asbestos, in the study by Musk *et al.* (2008) the exposure was predominantly to crocidolite, and in most of the other published studies that observed positive associations, populations were exposed to mixtures of different asbestos fibres.

The Working Group noted a positive association between exposure to abestos and cancer of the colorectum, based on the fairly consistent findings of the occupational cohort studies, plus the evidence for positive exposure–response relationships between cumulative asbestos exposure and cancer of the colorectum consistently reported in the more detailed cohort studies (McDonald *et al.*, 1980; Albin *et al.*, 1990; Berry *et al.*, 2000; Aliyu *et al.*, 2005). The conclusion was further supported by the results of four large and well performed meta-analyses (Frumkin & Berlin 1988; Homa *et al.*, 1994; IOM, 2006; Gamble, 2008).

Positive exposure–response relationships between asbestos exposure and cancer of the colorectum appear most likely to be seen in studies of populations with prolonged heavy exposure to asbestos that had long-term follow-up, and that incorporated high-quality assessments of exposure. The less detailed assessments of exposure found in many of the published studies would have tended to bias study results towards the null, and thus impede recognition of an association between asbestos exposure and cancer of the colorectum, even if such an association were truly present.

The apparently non-positive findings of several the case–control studies are not a deterrent to this conclusion. The majority of these case–control studies incorporated relatively little information on levels of asbestos exposure; indeed, most of them considered exposure as simply a dichotomous yes/no variable. Some of the case–control studies also may be compromised by inadequate duration of follow-up. Thus, the Garabrant study (Garabrant *et al.*, 1992) may be subject to the criticism, offered by Gerhardsson de Verdier *et al.* (1992) that "the highest duration of exposure…was 'at least 15 years,' a period that may be too short to detect an elevated risk."

There is some suggestion in the literature that the association between asbestos might be stronger for colon cancer than for rectal cancer. This view is supported by the meta-analysis of Gamble (2008) which found a positive dose–response relationship for cancer of the colorectum taken together, but not for rectal cancer. It is supported also by the study of Jakobsson *et al.* (1994), which found excess of cancer of the right colon in asbestos-exposed workers, but not of the left colon.

However, there was insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause cancer of the colorectum. It is of note in the study by McDonald *et al.* (1980) that exposure was to virtually pure chrysotile asbestos, whereas in most of the other studies cited above, populations were exposed to mixtures of different asbestos fibres.

3. Cancer in Experimental Animals

3.1 Introduction

Asbestos is a collective name for six different types of fibres: chrysotile, crocidolite, amosite, anthophyllite, tremolite, actinolite (see Section 1). Dusts from various deposits of the same type of asbestos can cause variations in the severity of the effects observed. Erionite is a fibrous zeolite found in Central Anatolia (Turkey), and Oregon (USA) (see Section 1 of the *Monograph* on Erionite). Talc is a hydrated magnesium silicate, and talc ore may contain several other minerals including anthophyllite, tremolite, calcite, dolomite, magnesite, antigorite, quartz, pyrophyllite micas, or chlorites (see Section 1).

The definition of pathogenic fibre properties as "sufficiently long, thin, and durable" is the subject of much debate, as are the differences between the exposure–response relationships or retained dose–response relationships of asbestos fibres in man and in rats, and the potential differences in the carcinogenicity of chrysotile compared to the various amphibole asbestos types. One of the reasons for a potential difference is a difference in the biopersistence between the two asbestos groups mentioned. The biopersistence is higher in the amphibole group (Hesterberg *et al.*, 1996, 1998a, b). The rat is the main test model for fibre-induced diseases. As the removal of asbestos fibres due to biosolubility is slow compared to the lifetime of rats and hamsters, experiments with this model may not be appropriate in predicting results of risk in humans (Berry, 1999).

Critical fibre dimensions to be used in toxicology and occupational regulations were discussed by the Working Group. It is generally agreed that the carcinogenic potency of a fibre increases with fibre length. Apart from the ongoing scientific view, standards of regulated fibres, with few exceptions, are based on the WHO fibre definition: aspect ratio ≥ 3: 1, length ≥ 5 µm, diameter ≤ 3 µm.

The tested materials (asbestos and erionite) are not presented in separate tables as in many cases they were tested in parallel experiments. The reason to split the inhalation studies into two tables (Table 3.1; Table 3.2) is that in many studies, various asbestos fibres were used as positive control in studies in which man-made fibres were tested (Table 3.2). In these latter studies, normally only one asbestos concentration was used. As for intrapleural and intraperitoneal studies, Table 3.4 is separate from Table 3.5 because the studies of Stanton *et al.* (1981) (see Table 3.5) included many fibre types – which also included fibres not to be reviewed here – and was designed to investigate the effect of fibre length and fibre type on mesothelioma induction.

A general evaluation on the type of fibre application in animal studies and an evaluation of some of the asbestos studies listed in Tables 3.1–3.5 can be found in Pott (1993) and IARC (2002).

3.2 Inhalation exposure

Table 3.1 and Table 3.2 give an overview of the numerous inhalation experiments on asbestos, and a few experiments on erionite. Some of these are described more extensively below.

Bronchial carcinomas and pleural mesotheliomas have been observed in rats after exposure to chrysotile, crocidolite, amosite, anthophyllite, and tremolite fibres. In these studies, there was no consistent increase in

tumour incidence at other sites. [The Working Group noted that in many studies, no complete histopathology was done.] All relatively short UICC asbestos preparations showed chronic effects in lung (based on fibre lenghts > 5 μm in the dust chamber) for fibres quantitatively roughly the same.

One of the first inhalation study with asbestos in rats that showed exposure–response relationships is the experiment of Wagner *et al.* (1974). Wistar rats were exposed to 10–15 mg/m^3 of one of the five UICC standard asbestos samples for 7 hours per day, mostly 5 days per week. The duration of exposure lasted from one day to 24 months. According to the reported data, in the group exposed to crocidolite for one day, lung tumours and one mesothelioma were found in 7/43 rats (16%). The corresponding exposure to chrysotile A (from Canada) resulted in lung tumours in 5/45 rats; for amosite 4/45 rats developed lung tumours and one mesothelioma. Three months of exposure to the five UICC standard asbestos samples resulted in the following thoracic tumour (mainly of the lung) incidences: chrysotile A, 44%;chrysotile B (from Zimbabwe), 53%; crocidolite, 42%; amosite, 27%; anthophyllite, 16%. Further results are listed in Table 3.1. In the 126 control rats, seven animals were also found to have lung tumours (Table 3.3). This high spontaneous lung tumour rate is a unique finding in Wistar rats. A review of unexposed control groups of many other studies shows that spontaneous lung tumours are very rare in this rat strain (Pott *et al.*, 1995; Table 3.3); on average, the incidence is less than one percent. Therefore, the very high tumour incidences described in this first inhalation study of Wagner *et al.* (1974) might be a misinterpretation of histopathological lesions because of a lack of experience at that time.

In a study conducted by Davis *et al.* (1978), five groups of Wistar rats were exposed to chrysotile (2.0, 10 mg/m^3), crocidolite (5.0, 10 mg/m^3), or amosite (10 mg/m^3). The highest tumour incidences (21–38%) were found in the chrysotile-exposed animals. This may be due to the relatively high fraction of fibres longer than 20 μm in the chrysotile dust used in this experiment. In addition to the lung tumours, extrapulmonary neoplasms included a relatively large number of peritoneal connective tissue tumours.

In a further study by Davis *et al.* (1986b), inhalation of short-fibred amosite did not produce tumours in Wistar rats (0/42). In contrast, there was a tumour incidence of 13/40 (33%) in a group exposed to long-fibred amosite. [The Working Group noted that extensive milling to produce short fibres may have altered the surface reactivity, see Section 4].

A group of 48 SPF Fischer rats was exposed to 10 mg/m^3 UICC chrysotile B by inhalation for 7 hours per day, 5 days per week, for 12 months (Wagner *et al.*, 1984b). This group served as positive controls in a study in which various man-made fibres were tested. After exposure, the animals were kept until natural death. Twelve thoracic tumours (one adenoma, 11 adenocarcinomas) were observed in 48 rats. In the untreated control group, no lung tumours were observed in 48 rats.

Smith *et al.* (1987) exposed groups of 58 female Osborne-Mendel rats to 7 mg/m^3 UICC crocidolite asbestos for 6 hours per day, for 5 days per week, for 2 years. After this treatment, rats were observed for life. The tumour incidence in rats exposed to crocidolite was 3/57 (one mesothelioma and two carcinomas). In the control group, no tumours were observed in 184 rats.

Special attention should be drawn to the crocidolite study with male Fischer rats of McConnell *et al.* (1994) because this study is very well documented. The exposure to 10 mg dust/m^3 (with 1610 WHO fibres/mL containing 236 fibres > 20 μm) for 6 h per day, 5 days per week had to be stopped after 10 months because of unexpected mortality, which was interpreted as a sign that the maximum tolerated dose had been exceeded. The number of WHO fibres per μg dry

Table 3.1 Studies of cancer in experimental animals exposed to various asbestos species and erionite (inhalation exposure)[a]

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Asbestos									
Chrysotile, Canada	86	NR	White rats 16 months or longer	6 h/d 5 d/wk 62 wk	0	10/41[c]	24		Gross *et al.* (1967)
Crocidolite	50	1105	Sprague-Dawley rats lifetime	4 h/d 4 d/w 24 mo	0	5/46	11		Reeves *et al.* (1974)
Chrysotile UICC/A	14.7	NR	Wistar rats lifetime	7 h/d 1 d	0	5/45	11		Wagner *et al.* (1974)
	12.3	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	16/36	44		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	8/19	42		
	10.9	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	19/27	70		
	10.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	11/17	65		

Table 3.1 (continued)

Test substance	Concentration (mg/m³)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC/B	9.7	NR	Wistar rats lifetime	7 h/d 1 d	0	1/42	2		
	12.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	18/34	53		
	10.2	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	5/17	29		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	3	14/23	61		
	10.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	1	11/21	52		
Crocidolite UICC	12.5	NR	Wistar rats lifetime	7 h/d 1 d	1	7/43	16		
	12.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	1	15/36	42		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	4/18	22		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	20/26	77		
	10.3	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	13/18	72		

Table 3.1 (continued)

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Amosite UICC	14.1	NR	Wistar rats lifetime	7 h/d 1 d	1	4/45	9		
	12.4	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	10/37	27		
	11.2	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	2/18	11		
	10.8	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	10/25	40		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	13/21	62		
Anthophyllite UICC	12.8	NR	Wistar rats lifetime	7 h/d 1 d	0	2/44	5		
	13.5	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	6/37	16		
	10.9	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	6/18	33		
	11.4	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	21/28	75		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	1	17/18	94		
Amosite UICC	10	550	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	2/43	5		Davis *et al.* (1978)

Table 3.1 (continued)

Test substance	Concentration (mg/m³)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Crocidolite UICC	5	430	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	3/43	7		
	10	860	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	1/40	3		
Chrysotile SFA	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	1	1/40	3		Wagner *et al.* (1980)
	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	4/18	22		
	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	8/22	36		
Chrysotile grade 7	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	0	1/39	3		
	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	5/18	28		
	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	3/24	13		
Chrysotile UICC (/B)	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	0	4/40	10		
	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	10/18	56		
	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	6/23	26		

Table 3.1 (continued)

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC /A	2	390	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	9/42	21		Davis *et al.* (1978)
Chrysotile UICC /A	10	1950	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	15/40	38		
Chrysotile UICC	9	NR	Wistar rats lifetime	7 h/d 1 d/wk 12 mo	0	6/43	14	Peak dosing (one d/ wk); no control group	Davis *et al.* (1980a)
Amosite UICC	50	NR	Wistar rats lifetime	7 h/d 1 d/w 12 mo	0	6/44	14	Peak dosing (one d/ wk); no control group	
Chrysotile UICC	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	15/43 (8 malignant, 7 benign)	35	No control group	Davis *et al.* (1980b)
Chrysotile "factory"	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	11/42 (3 maligant, 8 benign)	26	No control group	
Amosite "factory"	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	0/37	0	No control group	
Amosite UICC	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	2/40	5	No control group	
Tremolite	10	1600	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	20/39	51		Davis *et al.* (1985)
Crocidolite UICC	10	1630/350[d]	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	0	1/28	4		Wagner *et al.* (1985)
Chrysotile WDC textile yarn	3.5	679	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	18/41	44		Davis *et al.* (1986a)

Table 3.1 (continued)

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Chrysotile factory WDC	3.7	468	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	21/44	48		
Chrysotile textile yarn	3.5	428	Wistar rats lifetime	7 h/d 5 /wk 12 mo	1	16/42	38		
Chrysotile experimental WDC	3.5	108	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	4	21/43	49		
Chrysotile experimental WDC reversed daylight	3.8	111	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	18/37	49		
Amosite "long"	10	2060/1110[d]	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	13/40	33		Davis *et al.* (1986b)
Amosite "short"	10	70/12[d]	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	0/42	0		
Crocidolite UICC	10	NR	Fischer rats lifetime	6 h/d 5 d/wk 12 mo	0	1/28	4		Wagner *et al.* (1987)
Chrysotile, Canada, "long"	10	5510/1930[d]	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	22/40	55	1 peritoneal mesothelioma was observed in addition	Davis & Jones (1988)
Chrysotile, Canada, "short"	10	1170/330[d]	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	7/40	18	1 peritoneal mesothelioma was observed in addition	
Chrysotile UICC/A "discharged"	10	2670	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	11/39	28		Davis *et al.* (1988)

Table 3.1 (continued)

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC/A	10	2560	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	14/36	39		
Chrysotile UICC /A	10	2560	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	13/37	35		Davis *et al.* (1991a)
Chrysotile UICC /A	10	2545	Wistar rats lifetime	5 h/d 5 d/w 12 mo	2	26/41	63	Increase of tumour rate by particulate dust	
+ titanium dioxide	+ 10	-		+ 2 h/d 5 d/w 12 mo					
Chrysotile UICC /A	10	1960	Wistar rats lifetime	5 h/d 5 d/w 12 mo	6	22/38	58	Increase of tumour rate by particulate dust	
+ quartz S600	+ 2	-		+ 2 h/d 5 d/w 12 mo					
Amosite "long"	10	3648	Wistar rats lifetime	5 h/d 5 d/w 12 mo	2	20/40	50	Increase of tumour rate by particulate dust	Davis *et al.* (1991a)
+ titanium dioxide	+ 10	-		+ 2 h/d 5 d/w 12 mo					
Amosite "long"	10	4150	Wistar rats lifetime	5 h/d 5 d/w 12 mo	8	26/39	67	Increase of tumour rate by particulate dust	
+ quartz S600	+ 2	-		+ 2 h/d 5 d/w 12 mo					
Chrysotile Jeffrey	11	NR	Fischer rats lifetime	6 h/d 5 d/wk 12 mo	0	20/52	38		Mc Connell *et al.* (1991)

Table 3.1 (continued)

Test substance	Concentration (mg/m^3)	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[b]/ No. of animals examined	% tumours	Comments	Reference
Chrysotile	NR	NR	Baboons 6 yr	6 h/d 5 d/wk 4 years	0	0/6[e]	0		Goldstein & Coetzee (1990)
Crocidolite UICC	12-14	1130-1400	Baboons 6 yr	6 h/d 5 d/wk 4 yr	3	3/21[f]	14		
Amosite UICC	7	1110	Baboons 6 yr	6 h/d 5 d/wk 4 yr	2	2/11[f]	18		Goldstein & Coetzee (1990), Webster *et al.* (1993)
Erionite									
Erionite, Oregon	10	354	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	27	27/28	96		Wagner *et al.* (1985)
Erionite, Oregon	NR	NR	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	24	24/27	89	No control group	Wagner (1990)
Erionite, Oregon "short"	NR	NR	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	0	0/24	0	No control group	

[a] negative control groups: see Table 3.3

[b] Animals with benign or malignant lung tumour or pleural mesothelioma. The percentage of animals with tumours is related to the number of rats examined which were alive at a certain point in time (e.g. at the beginning of the experiment or after one year, or at the point in time of the death of the first animal with a tumour). Often, this is not clearly specified.

[c] observation time ≥6 mo

[d] Fibre count refers to fibres with lengths > 10 µm and diameters < 1 µm, in the aerosol

[e] observation time ≥4 yr

[f] observation time ≥5 yr

d, day or days; h, hour or hours; mo, month or months; NR, not reported; wk, week or weeks; yr, year or years

From Pott & Roller (1993b)

Table 3.2 Studies of cancer in experimental animals in which asbestos was used as positive control group (in inhalation studies of various man-made mineral fibres)

Test substance	Concentration (mg/m^3)	Aerosol fibres per cm^3 (L > 5 µm)	Species and strain (No. at risk); Observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[a] / No. of animals	% tumours	Comments	Reference
Amosite	NR	981 89 f > 20 µm/cm^3	AF/HAN rats, 24 mo	7 h/d 5 d/wk 12 mo	2	18/42 (7 carcinomas, 9 adenomas)	43		Davis *et al.* (1996), Cullen *et al.* (2000)
Chrysotile UICC/B	10	NR	Fischer rats, lifetime	7 h/d 5 d/wk 12 mo	0	11/56 (7 adenocarcinomas, 4 adenomas)	20		McConnell *et al.* (1984)
Chrysotile UICC/B	10	3832/1513[b]	Fischer rats, lifetime	7 h/d 5 d/wk 12 mo	0	12/48 (11 adenocarcinomas, 1 adenoma)	25		Wagner *et al.* (1984b)
Chrysotile NIEHS, Canada	10	10 600	Fischer rats, 24 mo	6 h/d 5 d/wk 24 mo	1	14/69	20		Hesterberg *et al.* (1993)
Crocidolite	10	1610	Fischer 344/N rats, 24 mo	6 h/d 5 d/wk 10 mo	1	14/106 (10 adenomas, 5 carcinomas)	13		McConnell *et al.* (1994)
Crocidolite UICC	7	3000/90[b]	Osborne-Mendel rats, lifetime	6 h/d 5 d/wk 24 mo	1	3/57 (1 mesothelioma, 2 carcinomas)	5		Smith *et al.* (1987)
Chrysotile UICC/A	Cumulative dose: 13 800 mg.h/m^3	NR	Rats, lifetime	6 h/d 5 d/wk 18 mo	0	9/39 (5 adenomas, 1 adenocarcinoma, 3 squamous cell carcinomas)	23	Strain not specified	Pigott & Ishmael (1982)
Amosite UICC	300	3090	Sprague-Dawley rats, 18–24 mo	6 h/d 5 d/wk 3 mo	0	3/16[c]	19	Small number of animals; D= 0.4 µm	Lee *et al.* (1981), Lee & Reinhardt (1984)
Chrysotile, Canada	5	5901	Wistar rats, 24 mo	5 h/d 5 d/wk 12–24 mo	0	9/47	19		Le Bouffant *et al.* (1987)
Chrysotile Calidria	6	131	Wistar rats, 24 mo	5 h/d 4 d/wk 12 mo	0	0/50	0		Muhle *et al.* (1987)

Table 3.2 (continued)

Test substance	Concentration (mg/m³)	Aerosol fibres per cm³ (L > 5 µm)	Species and strain (No. at risk); Observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours[a] / No. of animals	% tumours	Comments	Reference
Crocidolite, South Africa	2.2	162	Wistar rats, 24 mo	5 h/d 4 d/wk 12 mo	0	1/50	2		Muhle *et al.* (1987)
Amosite UICC	300	3090	Syrian golden hamsters, 18–24 mo	6 h/d 5 d/wk 3 mo	0	0/12	0	Small number of animals diameter, 0.4 µm	Lee *et al.* (1981), Lee & Reinhardt (1984)
Crocidolite UICC	7	3000/90[b]	Syrian golden hamsters, lifetime	6 h/d 5 d/wk 24 mo	0	0/58	0		Smith *et al.* (1987)
Amosite	0.8	36 WHO f/cm³ 10 f> 20 µm/cm³	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	3	3/83	3.6		McConnell *et al.* (1999)
	3.7	165 WHO f/cm³ 38 f> 20 µm/cm³	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	22	22/85	26		
	7.1	263 WHO f/cm³ 69 f> 20 µm/cm³	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	17	17/87	20		
Crocidolite UICC	13.5	1128	Baboons lifetime	7 h/d 5 d/wk 40 mo	0	0/10	0	All males	Goldstein *et al.* (1983)

[a] *n* = animals with benign or malignant lung tumour or pleural mesothelioma

[b] Number of fibres with a length > 10 µm and a diameter < 1 µm in the aerosol

d, day or days; f, fibre; h, hour or hours; mo, month or months; NR, not reported; RCF, refractory ceramic fibre; wk, week or weeks

From Pott & Roller (1993b)

Table 3.3 Negative controls (clean air for lifetime) in carcinogenicity studies after inhalation exposures from Table 3.1 and Table 3.2

Species and strain	Number of pleural mesothelioma	No. of animals with thoracic tumours[a]/ No. of animals	Reference
Fischer rats	0	0/48	Wagner *et al* (1984b)
Fischer rats	0	0/28	Wagner *et al.* (1985)
Fischer rats	0	0/28	Wagner *et al.* (1987)
Fischer rats	0	1/56	McConnell *et al.* (1991)
Fischer rats	0	4/123	Hesterberg *et al.* (1993)
Fischer rats	0	2/126	McConnell *et al.* (1994)
Osborne-Mendel rats	0	0/184	Smith *et al.* (1987)
Sprague-Dawley rats	0	1/5	Reeves *et al.* (1974)
Sprague-Dawley rats	0	0/19	Lee *et al.* (1981)
White rats	0	0/25	Gross *et al.* (1967)
Wistar rats	0	7/126	Wagner *et al.* (1974)
Wistar rats	0	0/20	Davis *et al.* (1978)
Wistar rats	0	1/71	Wagner *et al.* (1980)
Wistar rats	0	0/36	Davis *et al.* (1985)
Wistar rats	0	2/39	Davis *et al.* (1986a)
Wistar rats	0	0/25	Davis *et al.* (1986a)
Wistar rats	0	0/110	Muhle *et al.* (1987)
Wistar rats	0	2/36	Davis *et al.* (1988)
Wistar rats	0	0/25	Davis *et al.* (1988)
Wistar rats	0	2/47	Davis & Jones (1988)
Wistar rats	0	2/47	Davis *et al.* (1991a)
Syrian golden hamsters	0	1/170	Smith *et al.* (1987)
Syrian golden hamsters	0	0/83	Mc Connell *et al.* (1999)

[a] *n* = animals with benign or malignant lung tumour or pleural mesothelioma

lung tissue was 1850 (73 fibres > 20 µm) at the end of exposure and 759 WHO fibres (41 fibres > 20 µm) 12 months later. Fourteen out of 106 rats (13.2%), which survived the second year or longer, died with lung tumour (five of these rats developed lung carcinomas), and one rat also developed a mesothelioma. In the control group, 2/126 rats developed lung adenomas.

In two lifetime studies, male and female Fischer rats were exposed to either 10 mg/m^3 erionite (Wagner *et al.*, 1985) or an unknown concentration of erionite (Wagner, 1990) for 6 hours per day, 5 days per week, for 12 months. Twenty seven out of 28 rats, and 24/27 rats developed pleural mesotheliomas, respectively. No lung tumours were observed. [The Working Group noted the lack of control group in the study by Wagner (1990).]

McConnell *et al.* (1999) exposed three groups of 125 male Syrian golden hamsters to 0.8, 3.7 and 7.1 mg/m^3 amosite for 6 hours per day, 5 days per week, for 78 weeks. They were then held unexposed for 6 weeks. Among animals that survived for at least 32 weeks, 3/83, 22/85 and 17/87 developed pleural mesotheliomas, respectively. No mesotheliomas were observed in 83 untreated controls and no lung tumours were observed in any groups.

Some experiments were reported with baboons. After amosite exposure and crocidolite exposure for 4 years, 2/11 baboons and 3/21 baboons developed pleural mesothelioma,

respectively (Goldstein & Coetzee, 1990; Webster *et al.*, 1993).

3.3 Intrapleural and intraperitoneal administration

Animal experiments had shown that an intrapleural injection of a suspension of asbestos dusts in rats leads to mesotheliomas (Wagner, 1962; Wagner & Berry, 1969). The serosa has subsequently been taken as a model for the examination of the carcinogenicity of fibrous dusts in numerous studies. Some groups have opted for administration into the pleural cavity, others preferring intraperitoneal injection of dust suspensions. In comparison with the intrapleural model, the intraperitoneal carcinogenicity test on fibres has proven to be the method with the far greater capacity and, consequently, the greater sensitivity (see also Pott & Roller, 1993a). Results from these numerous experiments using asbestos and erionite are listed in Table 3.4.

Table 3.5 contains a summary of the experiments by Stanton *et al.* (1981). In this extensive study, the authors implanted 72 dusts containing fibres of various sizes in the pleura of Osborne-Mendel rats. The probability of the development of pleural mesotheliomas was highest for fibres with a diameter of less than 0.25 µm and lengths greater than 8 µm.

In summary, samples of all six asbestos types and of erionite were administered to rats by intrapleural or intraperitoneal injection in numerous studies. Consistently, mesothelima induction was observed when samples contained a sufficient fibre number with a fibre length > 5 µm.

3.4 Intratracheal administration

Only a few studies have been carried out with intratracheal instillation of asbestos fibres in rats (Pott *et al.*, 1987; Smith *et al.*, 1987), and hamsters (Pott *et al.*, 1984; Feron *et al.*, 1985; Smith *et al.*, 1987). Principally, in this experimental model, asbestos fibres induced lung tumours in rats, and lung tumours and mesotheliomas in hamsters. Studies in hamsters are described below.

In a 2-year study, a group of male Syrian golden hamsters [initial number unspecified] was intratracheally instilled with 1 mg UICC crocidolite in 0.15 mL saline once a week for 8 weeks. At the end of the experiment, the incidences of lung carcinomas and of pleural mesotheliomas were 9/142 [$P < 0.01$] and 8/142 [$P < 0.01$], respectively. No thoracic tumours were observed in 135 titanium-dioxide-treated control animals (Pott *et al.*, 1984).

In a lifetime study, a group of Syrian golden hamsters [sex and initial number unspecified] was intratracheally instilled with 2 mg UICC crocidolite in 0.2 mL saline once a week for 5 weeks. At the end of the experiment, 20/27 animals developed broncho-alveolar tumours ($p<0.05$), including 7/27 with malignant tumours [$p<0.05$]. No broncho-alveolar tumours were observed in 24 saline-treated controls (Smith *et al.*, 1987).

3.5 Oral administration

A study on the carcinogenicity of ingested asbestos fibres involved male F344 rats groups exposed to amosite or chrysotile in combination with subcutaneous administration of a known intestinal carcinogen, azoxymethane (10 weekly injections of 7.4 mg/kg body weight). Fibres were administered three times a week for 10 weeks by intragastric bolus dosing (10 mg in 1 mL saline). The first experiment in this study included a full set of appropriate control groups. The experiment was terminated at 34 weeks. Neither amosite nor UICC chrysotile B, in combination with azoxymethane, increased the incidence of any intestinal tumours (≈10%) above that produced by azoxymethane alone, but the combination with either fibre type produced 4–5-fold increases

(not significant, $P > 0.1$) in metastatic intestinal tumours. A second experiment with larger groups, the same dosing regimen, and for lifetime, but with a more limited design, tested only amosite in combination with azoxymethane versus azoxymethane. Amosite did not enhance azoxymethane-induced intestinal tumours (incidence, 77% versus 67%) (Ward *et al.*, 1980; IOM, 2006). [The Working Group noted that the lack of untreated vehicle controls in the second experiment made interpretation of the results difficult considering that, compared to historical controls, there was a non-significant increase in intestinal tumours in rats exposed only to amosite (≈33%). One cannot know whether the results observed were associated with the asbestos or with irritation from the procedure, although one would not anticipate that gavage itself would impact the lower portion of the gastrointestinal tract.]

The most definitive animal studies of oral exposure to asbestos were a series of lifetime studies conducted by the National Toxicology Program (NTP, 1983, 1985, 1988, 1990a, b), in which asbestos (chrysotile, crocidolite, and amosite) was administered in the feed of rats and hamsters. Nonfibrous tremolite was also tested in rats according to the same protocol (NTP, 1990c). Exposure of dams of the study animals (1% in the diet) was followed by exposure of the pups by gavage (0.47 mg/g water) while they were nursing, and then in the diet for the remainder of their lives: they were exposed to asbestos at the level of 1%, which was estimated by the investigators to be about 70000 times the greatest possible human exposure in drinking-water. Histopathological examination of the entire colorectum was performed. No increases in the incidence of gastrointestinal lesions (inflammatory, preneoplastic, or neoplastic) were found after exposure to intermediate-length chrysotile (from Quebec) in hamsters, to short chrysotile (from New Idria) in rats or hamsters, to amosite in rats or hamsters, to crocidolite in rats, or to non-fibrous tremolite in rats. The mesentery was examined in detail, as well as mesenteric lymph nodes and sections of the larynx, trachea, and lungs from every animal. No lesions were found in any of those tissues. The only finding of note in the gastrointestinal tract was a slight increase in the incidence of adenomatous polyps in the large intestine after exposure to the intermediate-length chrysotile (from Quebec) in male rats (9/250 versus 0/85, $P = 0.08$), but preneoplastic changes in the epithelium were not found (NTP, 1985; IOM, 2006).

3.6 Intragastric administration

White rats, 2–3 months old, were surgically applied, on the greater curvature of the stomach, a perforated capsule containing 0 (control) or 100 mg chrysotile asbestos in a filler (beef fat: natural wax, 1:1). Tumours observed in 18/75 asbestos-exposed rats, between 18–30 months after the beginning of the experiment, were the following: eight gastric adenomas, two gastric adenocarcinomas, one gastric carcinoma, one cancer of the forestomach, one small intestine adenocarcinoma, two peritoneal mesotheliomas, and three abdominal lymphoreticular sarcomas. No tumours were observed in 75 control animals (Kogan *et al.*, 1987). [The Working Group noted various unresolved questions regarding the design of this study in particular the very high dose of 100 mg.]

3.7 Studies in companion animals

Mesotheliomas were reported in pet dogs with asbestos exposure in the households of their owners. Eighteen dogs diagnosed with mesothelioma and 32 age-, breed- and gender-matched control dogs were investigated. Sixteen owners of cases and all owners of controls were interviewed. An asbestos-related occupation or hobby of a household member was significantly associated with mesothelioma observed in cases (OR,

Table 3.4 Studies of cancer in rats exposed to asbestos fibres and erionite (intrapleural and intraperitoneal administration)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres [a] [10^9]	Tumour incidence [b]		Significance [c]	Comments
					n/z	%		
Asbestos	**Asbestos type**							
Wistar – Pott *et al.* (1989)	Actinolite	0.25	i.p.	0.1	20/36	56	***	
Wistar – Wagner *et al.* (1973)	Amosite UICC	20	i.pl.	NR	11/32	34	***	
Wistar – Davis *et al.* (1991b)	Amosite from UICC	0.01	i.p.	0.0003	4/48	8	*	
Wistar – Davis *et al.* (1991b)	Amosite from UICC	0.05	i.p.	0.002	8/32	25	***	
Wistar – Davis *et al.* (1991b)	Amosite from UICC	0.5	i.p.	0.02	15/32	47	***	
Wistar – Wagner *et al.* (1973)	Anthophyllite UICC	20	i.pl.	NR	8/32	25	***	
Wistar – Wagner *et al.* (1973)	Chrysotile UICC/A	20	i.pl.	NR	7/31	23	***	
Sprague-Dawley – Monchaux *et al.* (1981)	Chrysotile UICC/A	20	i.pl.	NR	14/33	42	***	
Sprague-Dawley – Wagner *et al.* (1984b)	Chrysotile UICC/A	20	i.pl.	19.6	6/48	13	**	
Wistar – Pigott & Ishmael (1992)	Chrysotile UICC/A	20	i.pl.	NR	7/48	15	***	
Fischer – Coffin *et al.* (1992)	Chrysotile UICC/A	0.5	i.pl.	0.90	118/142[d]	78	***[d]	
		2		3.6		87		
		4		7.2		92		
		8		14		83		
		16		29		83		
		32		57		75		
Wistar – Wagner *et al.* (1973)	Chrysotile UICC/B	20	i.pl.	NR	10/32	31	***	
Wistar – Wagner *et al.* (1980)	Chrysotile UICC/B	20	i.pl.	NR	5/48	10	*	
Fischer – Wagner *et al.* (1987)	Chrysotile UICC/B	20	i.pl.	NR	19/39	49	***	
Wistar – Pott *et al.* (1989)	Chrysotile UICC/B	0.25	i.p.	0.2	23/34	68	***	

Table 3.4 (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres [a] [10^9]	Tumour incidence [b]		Significance [c]	Comments
					n/z	%		
Wistar – Davis *et al.* (1991b)	Chrysotile from UICC/A	0.01	i.p.	0.002	2/48	4	NS	
Wistar – Davis *et al.* (1991b)	Chrysotile from UICC/A	0.05	i.p.	0.009	12/32	38	***	
Wistar – Davis *et al.* (1991b)	Chrysotile from UICC/A	0.5	i.p.	0.09	26/32	81	***	
Wistar – Wagner *et al.* (1973)	Crocidolite UICC	20	i.pl.	NR	19/32	59	***	
Fischer – Wagner *et al.* (1987)	Crocidolite UICC	20	i.pl.	NR	34/40	85	***	
Fischer – Wagner (1990)	Crocidolite UICC	20	i.pl.	NR	24/32	75	***	
Sprague-Dawley – Monchaux *et al.* (1981)	Crocidolite UICC	20	i.pl.	NR	21/39	54	***	
Osborne-Mendel – Stanton *et al.* (1981)	Crocidolite UICC	40	i.pl.	NR	14/29	48	***	
Fischer – Wagner *et al.* (1984a)	Crocidolite UICC	20	i.pl.	NR	35/41	85	***	
Fischer – Wagner *et al.* (1984a)	Crocidolite UICC ground 1 h	20	i.pl.	NR	34/42	81	***	
Fischer – Wagner *et al.* (1984a)	Crocidolite UICC ground 2 h	20	i.pl.	NR	34/42	81	***	
Fischer – Wagner *et al.* (1984a)	Crocidolite UICC ground 4 h	20	i.pl.	NR	15/41	37	***	
Fischer – Wagner *et al.* (1984a)	Crocidolite UICC ground 8 h	20	i.pl.	NR	13/42	31	***	
Fischer – Coffin *et al.* (1992)	Crocidolite UICC	0.5	i.pl.	0.04	65/144[d]	29	** [d]	
		2		0.16		13		
		4		0.32		50		
		8		0.65		67		
		16		1.3		58		
		32		2.6		54		
Wistar – Davis *et al.* (1991b)	Crocidolite from UICC	0.01	i.p.	0.0004	0/48	0	NS	
Wistar – Davis *et al.* (1991b)	Crocidolite from UICC	0.05	i.p.	0.002	8/32	25	***	

Table 3.4 (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres [a] [10^9]	Tumour incidence [b]		Significance [c]	Comments
					n/z	%		
Wistar – Davis *et al.* (1991b)	Crocidolite from UICC	0.5	i.p.	0.02	10/32	31	***	
Wistar – Pott *et al.* (1987)	Crocidolite South Africa	0.5	i.p.	0.05	18/32	56	***	
Wistar – Roller *et al.* (1996)	Crocidolite A	0.5	i.p.	0.042	25/32	78	***	All females
Wistar – Roller *et al.* (1996)	Crocidolite A	0.5	i.p.	0.042	32/48	67	***	All females
Wistar – Roller *et al.* (1996)	Crocidolite C	0.5	i.p.	0.042	20/39	51	***	
Wistar – Davis *et al.* (1985)	Tremolite, Korea	25	i.p.	NR	27/29	93	***	
Wistar – Roller *et al.* (1996)	Tremolite B	3.3	i.p.	0.057	9/40	23	***	
Wistar – Roller *et al.* (1996)	Tremolite B	15	i.p.	0.26	30/40	75	***	
Erionite	Erionite type							
Sprague-Dawley – Pott *et al.* (1987)	Karain	1.25	i.p.	NR	38/53	72	***	
Sprague-Dawley – Pott *et al.* (1987)	Karain	5	i.p.	NR	43/53	81	***	
Sprague-Dawley – Pott *et al.* (1987)	Karain	20	i.p.	G	37/53	70	***	
Fischer – Wagner *et al.* (1985)	Karain	20	i.pl.	NR	38/40	95	***	
Fischer – Wagner *et al.* (1985)	Oregon	20	i.pl.	NR	40/40	100	***	
Wistar – Pott *et al.* (1987)	Oregon	0.5	i.p.	0.02	15/31	48	***	
Wistar – Pott *et al.* (1987)	Oregon	2	i.p.	0.08	28/31	90	***	
Fischer – Wagner (1990)	Oregon	20	i.pl.	NR	30/32	94	***	
Fischer – Wagner (1990)	Oregon “short”	20	i.pl.	NR	0/32	0	NS	
Wistar – Davis *et al.* (1991b)	Oregon	0.005	i.p.	0.00025	0/48	0	NS	
		0.01		0.0005	4/48	8	*	
		0.05		0.0025	15/32	47	***	
		0.5		0.025	26/32	81	***	
		2.5		0.125	30/32	94	***	
		5		0.25	21/24	88	***	
		10		0.5	20/24	83	***	
		25		1.25	17/18	94	***	

Table 3.4 (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres [a] [10^9]	Tumour incidence [b]		Significance [c]	Comments
					n/z	%		
Porton – Hill *et al.* (1990)	Oregon	0.1	i.pl.	NR	5/10	50	*	
		1		NR	9/10	90	***	
		10		NR	9/10	90	***	
		20		NR	8/10	80	***	
Wistar – Kleymenova *et al.* (1999)	Grusia mines	20	i.pl.	NR	39/40	98	?	
Fischer – Coffin *et al.* (1992)	Oregon "C"	0.5	i.pl.	NR	123/144[d]	79	***[d]	
		2		NR		87		
		4		NR		83		
		8		NR		84		
		16		NR		87		
		32		NR		91		
Fischer – Coffin *et al.* (1992)	Oregon "W"	0.5	i.pl.	NR	137/144[d]	100	***[d]	
		2		NR		92		
		4		NR		100		
		8		NR		91		
		16		NR		96		
		32		NR		92		
Sprague-Dawley – Maltoni & Minardi (1989)	"Sedimentary erionite"	25	i.pl.	NR	35/40	88	***	
Sprague-Dawley – Maltoni & Minardi (1989)	"Sedimentary erionite"	25	i.p.	NR	35/40	50	***	

[a] The fibre numbers mainly refer to fibres with a length greater than 5 µm
[b] n/z number of animals with serosal tumour (mesothelioma/sarcoma) / number of animals examined
[c] calculation of the statistical significance with the Fisher exact test, one-sided: *** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$
[d] combined data of 6 groups
i.p., intrapleural; i.pl., intraperitoneal; NS, not significant; NR, not reported
From Pott & Roller (1993b)

Table 3.5 Carcinogenicity study of intrapleural application of asbestos fibres and other fibrous materials in female Osborne-Mendel rats (40 mg fibres per rat)

Fibrous dust (material)	No. of fibres [a] (x10^6) L > 8 µm D < 0.25 µm	Probability of pleural sarcomas [b]		Pleural sarcoma incidence [c]	
				n/z	%
Tremolite 1	55	100		22/28	79
Tremolite 2	28	100		21/28	75
Crocidolite 1	6500	94	± 6.0	18/27	67
Crocidolite 2	800	93	± 6.5	17/24	71
Crocidolite 3	4100	93	± 6.9	15/23	65
Amosite	140	93	± 7.1	14/25	56
Crocidolite 4	5400	86	± 9.0	15/24	63
Crocidolite 5 (UICC)	78	78	± 10.8	14/29	48
Crocidolite 6	1600	63	± 13.9	9/27	33
Crocidolite 7	18	56	± 11.7	11/26	42
Crocidolite 8	< 0.3[d]	53	± 12.9	8/25	32
Crocidolite 9	710	33	± 9.8	8/27	30
Crocidolite 10	49	37	± 13.5	6/29	21
Crocidolite 11	< 0.3[d]	19	± 8.5	4/29	14
Crocidolite 12	220	10	± 7.0	2/27	7
Talc 1	< 0.3[d]	7	± 6.9	1/26	4
Talc 3	< 0.3[d]	4	± 4.3	1/29	3
Talc 2	< 0.3[d]	4	± 3.8	1/30	3
Talc 4	< 0.3[d]	5	± 4.9	1/29	3
Crocidolite 13	< 0.3[d]	0		0/29	0
Talc 5	< 0.3[d]	0		0/30	0
Talc 6	80	0		0/30	0
Talc 7	< 0.3[d]	0		0/29	0

[a] Fibre numbers stated in original work as common logarithm.

[b] Calculation taking into account the different life spans (life table method).

[c] n/z = number of rats with pleural sarcomas/number of rats examined. Frequency of pleural sarcomas in female control rats: untreated, 3 animals out of 491 (0.6%); with non-carcinogenic lung implantates, 9 out of 441 (2.0%); with non-carcinogenic pleural implantates, 17 out of 615 (2.8%). [17 out of 615 against 3 out of 491, according to Fisher exact test$P < 0.01$]. All three control groups are brought together by Stanton *et al.* (1981) to 29 out of 1518 animals (1.9%); for this after application of the life table method a tumour probability of 7.7 ± 4.2% is indicated. [Without any reason being given it is concluded that the tumour probability in any one of the groups treated according to the life table method must exceed 30% to be "significantly" increased.] Significance limit for Fisher test in the case of 25 to 30 animals against 17 out of 615 control rats: approx. 12 to 13% tumour frequency. (The term "tumour frequency" is not to be equated with tumour probability according to the life table method. The "significance limit" of 30% mentioned by Stanton *et al.* (1981) refers to life table incidence or probability.

[d] The de-logarithmised fibre numbers with the above mentioned definition are between 0 and 0.3.

From Stanton *et al.* (1981)

8.0; 95%CI: 1.4–45.9). Lung tissue from three dogs with mesothelioma and one dog with squamous cell carcinoma of the lung had higher level of chrysotile asbestos fibres than lung tissue from control dogs (Glickman *et al.*, 1983).

3.8 Synthesis

Bronchial carcinomas and pleural mesotheliomas were observed in many experiments in rats after exposure to chrysotile, crocidolite, amosite, anthophyllite, and tremolite fibres. In these studies, there was no consistent increase in tumour incidence at other sites. A special preparation of "long" crocidolite was more effective to induce lung tumours compared to the "short" UICC asbestos samples on the basis of administered dose in f/mL.

In one study in Syrian golden hamsters with three different concentrations of amosite, a significant increase in pleural mesothelioma incidence was observed, but no lung tumours were found.

After amosite exposure and crocidolite exposure by inhalation, 2/11 baboons and 3/21 baboons developed pleural mesothelioma, respectively.

In two studies in rats exposed to erionite, a significant increase in pleural mesothelioma incidence was observed. However, no lung tumours were found.

Samples of all six asbestos types and of erionite were administered to rats by intrapleural or intraperitoneal injection in numerous studies. Consistently, mesothelioma induction was observed when samples contained a sufficient fibre number with a fibre length > 5 µm.

Only a few studies have been carried out with intratracheal instillation of crocidolite in rats and hamsters. Malignant lung tumours were observed in rats, and pleural mesothelioma and malignant lung tumours were observed in hamsters.

Chrysolite, crocidolite and amosite were administered in the feed of rats and hamsters. No increase of the incidence of gastrointestinal tumours was observed in both species.

No chronic studies with vermiculite containing asbestos fibres or talc containing asbestos fibres could be identified.

4. Other Relevant Data

4.1 Toxicokinetics, deposition, clearance, and translocation in humans

4.1.1 Aerodynamic and anatomical factors

Inhalation is the most important route of exposure to mineral fibres, and is associated with the development of non-malignant diseases of the lungs and pleura, and malignant diseases arising in the lung, larynx, and pleural and peritoneal linings (IOM, 2006). The deposition of particles and fibres in the lungs is dependent on their aerodynamic diameter, which is a function of geometry, aspect ratio (IARC, 2002), and density (Bernstein *et al.*, 2005). Fibres can deposit by sedimentation, by impaction at bronchial bifurcations or by interception of the fibre tip with the bronchial wall. Smaller diameter fibres are likely to deposit in the alveoli (Bernstein *et al.*, 2005).

Particles and fibres can be cleared from the nasal and tracheobronchial regions by mucociliary transport (Lippmann *et al.*, 1980). Following deposition in the distal airways and alveoli, short fibres are removed more slowly following phagocytosis by alveolar macrophages. Fibre length is a limiting factor in macrophage-mediated clearance; fibres longer than the diameter of human alveolar macrophages (approximately 14–25 µm) are less likely to be cleared. Fibres may also interact with lung epithelial cells, penetrate into the interstitium, and translocate to the pleura and peritoneum or more distant sites. Fibres that are not efficiently cleared or altered by physicochemical process (e.g. breakage, splitting, or

chemical modification) are termed biopersistent (Bernstein *et al.*, 2005). Chronic inhalation assays using man-made fibres in rodents have correlated fibre length and biopersistence with persistent inflammation, fibrosis, lung cancer, and malignant mesothelioma (Bernstein *et al.*, 2005). However, there are interspecies differences in alveolar deposition of inhaled particles and fibres that must be considered when extrapolating results of rodent inhalation studies to humans (IARC, 2002).

4.1.2 Biopersistence of asbestos and erionite fibres

Asbestos fibres and ferruginous bodies (described subsequently in Section 4.3.1) can be identified and quantified by tissue digestion of lung samples obtained by biopsy or at autopsy (Roggli, 1990). A variety of commercial and non-commercial asbestos fibres have been identified in residents older than 40 years of age living in an urban area with no history of occupational asbestos exposure (Churg & Warnock, 1980). These and other studies confirm that asbestos fibres are biopersistent and accumulate in lung tissue as well as lymph nodes (Dodson *et al.*, 1990; Dodson & Atkinson, 2006). Asbestos fibres have also been identified in the pleura following autopsy (Dodson *et al.*, 1990; Gibbs *et al.*, 1991; Suzuki & Yuen, 2001) and in the parietal pleural in samples collected during thoracoscopy (Boutin *et al.*, 1996). Roggli *et al.* (1980) also identified asbestos bodies in the larynx of asbestos workers at autopsy. Systemic translocation of asbestos fibres to distant organs has also been described in case reports; however, these reports should be evaluated with caution due to the numerous caveats in technical procedures used, comparison with an appropriate control population, and cross-contamination of tissue samples (Roggli, 2006). The route of translocation of asbestos fibres from the lungs to distant sites is unknown, although lymphatic translocation of amosite fibres deposited in the lungs has been shown in experimental animals (Hesterberg *et al.*, 1999; Mc Connell *et al.*, 1999; IOM, 2006; NIOSH, 2009).

Environmental exposure to erionite fibres is associated with diffuse malignant mesothelioma in three rural villages in the Cappadocia region of Turkey (Baris & Grandjean, 2006). Lung fibre digests obtained from humans in these villages showed elevated levels of erionite fibres, and ferruginous bodies surrounding erionite fibres were found in broncho-alveolar lavage fluid (Sébastien *et al.*, 1984; Dumortier *et al.*, 2001).

Talc particles have been found in the lungs at autopsy of both rural and urban residents as well as talc miners (IARC, 1987b, 2010). Talc particles are biopersistent in the lungs, and have been recovered in broncho-alveolar lavage fluid obtained from workers 21 years after cessation of occupational exposure (Dumortier *et al.*, 1989). Talc contaminated with asbestos has been linked to the development of lung cancer and malignant mesothelioma (IARC, 1987b).

The association between exposure to talc, potential retrograde translocation to the ovarian epithelium, and the development of ovarian cancer is controversial (IARC, 2010, and this volume).

The biological plausibility for an association between asbestos and ovarian cancer derives in part from the finding of asbestos fibres in the ovaries of women with potential for exposure to asbestos. Thus, a histopathological study of ovaries from 13 women who had household contact with men who had documented exposure to asbestos, and of 17 women who gave no history of potential for asbestos exposure found "significant asbestos fibre burdens" in the ovaries of nine (60.2%) of the exposed women and in only six (35%) of the unexposed women. Three of the exposed women had asbestos fibre counts in ovarian tissue of over 1 million fibres per gram (wet weight), but only one of the 17

women without exposure had counts in that range (Heller *et al.*, 1996).

Further support for the biological plausibility of an association between asbestos exposure and ovarian cancer derives from an experimental study (Graham & Graham, 1967) that found that the intraperitoneal injection of tremolite asbestos into guinea-pigs and rabbits produced epithelial changes in the ovaries "similar to those seen in patients with early ovarian cancer".

[The Working Group noted that the histopathological diagnosis of ovarian carcinoma is difficult and requires the application of immunohistochemical techniques to distinguish between this cancer and peritoneal malignant mesothelioma. These techniques and the recognition of borderline ovarian tumours and variants of serosal tumours that arise in the pelvis of women were not applied in the Graham & Graham study in 1967. In addition, mesothelial hyperplasia occurs commonly in the pelvic region, and is not considered a preneoplastic lesion (NIOSH, 2009).]

4.2 Molecular pathogenesis of human cancers related to mineral dust exposure

Cancers develop in the upper and lower respiratory tract (carcinoma of the larynx and lungs), and in the pleural and peritoneal linings (diffuse malignant mesothelioma) after a long latent period up to 20–40 years following initial exposure to asbestos or erionite fibres (IARC, 1977; IOM, 2006). During the long latent period before the clinical diagnosis of cancer of the lung or of the larynx or diffuse malignant mesothelioma, multiple genetic and molecular alterations involving the activation of cell growth regulatory pathways, the mutation or amplification of oncogenes, and the inactivation of tumour–suppressor genes characterize specific histopathological types of these tumours that have been associated with exposure to mineral dust or fibres. Some of these molecular alterations have been linked to specific chemical carcinogens in tobacco smoke (Nelson & Kelsey, 2002), and additional alterations may arise secondarily due to chronic inflammation, genetic instability, or epigenetic changes that will be discussed in detail in Section 4.3.

Additional pathways related to resistance to apoptosis, acquired genetic instability, and angiogenesis are activated or upregulated during the later stages of tumour progression of lung cancer and diffuse malignant mesothelioma (Table 4.1; Table 4.2). No mutations in oncogenes or tumour-suppressor genes have been directly linked with exposure to asbestos fibres (NIOSH, 2009).

4.2.1 Cancer of the lung and of the larynx

Lung cancers are classified into two histological subtypes: small cell carcinoma and non-small cell carcinoma (Table 4.1). In non-small cell lung carcinoma, activating point mutations in the *K-RAS* oncogene have been linked to specific chemical carcinogens in tobacco smoke; Nelson *et al.* (1999) described more frequent *K-RAS* mutations in lung carcinomas in asbestos-exposed workers. Loss of heterozygosity and point mutations in the *p53* tumour-suppressor gene have also been linked with tobacco smoke carcinogens in cancer of the lung and of the larynx (Pfeifer *et al.*, 2002; NIOSH, 2009). These alterations have also been described in lung cancers in asbestos-exposed workers (Nymark *et al.*, 2008).

4.2.2 Diffuse malignant mesothelioma

Malignant tumours arising in the pleural or peritoneal linings (diffuse malignant mesothelioma) have no association with tobacco smoking, and are characterized by a different spectrum of molecular alterations (Table 4.2). In contrast with lung cancers associated with tobacco smoking and asbestos exposure, mutations in the *K-RAS*

Table 4.1 Some reported molecular alterations in bronchogenic carcinoma

Functional alterations	Gene target	Histological type of lung cancer	
		Small cell	Non-small cell
Autocrine growth stimulation	Growth factors and receptors	GRP/GRP receptor SCF/KIT	TGF-α/EGFR HGF/MET
Activation of oncogenes	*RAS* mutation	<1%	15–20%
	MYC overexpression	15–30%	5–10%
Inactivation of tumour-suppressor genes	*p53* mutation	~90%	~50%
	RB mutation	~90%	15–30%
	$p16^{INK4A}$ inactivation	0–10%	30–70%
	FHIT inactivation	~75%	50–75%
Resistance to apoptosis	*BCL2* expression	75–95%	10–35%
Genetic instability	Microsatellite instability	~35%	~22%

EGFR, epidermal growth factor receptor; FHIT, fragile histidine triad; GRP, gastrin-releasing peptide; HGF, hepatocyte growth factor; RB, retinoblastoma gene; SCF, stem cell factor; TGF-α, transforming growth factor-α.
From Sekido *et al.* (2001), Sato *et al.* (2007), Schwartz *et al.* (2007), NIOSH (2009)

oncogene or the *p53* tumour-suppressor gene are rare. The most frequent molecular alteration involves deletion or hypermethylation at the *CDKN2A/ARF* locus on chromosome 9p21 which contains three tumour-suppressor genes: *p15*, *p16* ^{INK4A}, and *p14* ^{ARF} (Murthy & Testa, 1999). Additional molecular alterations include hypermethylation and silencing of the *RASSFIA* and *GPC3* tumour-suppressor genes, and inactivation of the *NF2* tumour-suppressor gene (Apostolou *et al.*, 2006; Murthy *et al.*, 2000).

Comparative genomic hydrizidation, gene expression profiling, and proteomics have been used to identify specific diagnostic and prognostic biomarkers for diffuse malignant mesothelioma (Wali *et al.*, 2005; Greillier *et al.*, 2008). The most promising outcome of these global screening strategies is the identification of two potential serum or pleural fluid biomarkers that may provide early diagnosis of malignant pleural mesothelioma: osteopontin (Pass *et al.*, 2005), and soluble mesothelin-related protein (Robinson *et al.*, 2005). Both of these markers have been shown to be elevated in most patients diagnosed with diffused malignant mesothelioma, but are not entirely specific for these cancers (Greillier *et al.*, 2008). No gene expression signature can be attributed directly to asbestos exposure, and these studies show variable gene expression patterns resulting from limited stability of RNA, contamination of tumour samples with host cells, and use of different microarray platforms (López-Ríos *et al.*, 2006).

In addition to the genetic and chromosomal alterations that have been identified in diffuse malignant mesothelioma (Table 4.2), epigenetic alterations characterized by altered patterns of DNA methylation have been described (Toyooka *et al.*, 2001; Tsou *et al.*, 2005). Overall, human tumours have been characterized by global hypomethylation associated with hypermethylation of CpG islands in the promoter regions of tumour-suppressor genes leading to their inactivation. These alterations in DNA methylation are the most common molecular or genetic lesion in human cancer (Esteller, 2005). Recent comprehensive analyses of epigenetic profiles of 158 patients with malignant pleural mesotheliomas and 18 normal pleural samples using 803 cancer-related genes revealed classes of methylation profiles in malignant mesothelioma that were associated with asbestos lung burden and survival (Christensen *et al.*, 2009). Other data confirmed hypermethylation of cell-cycle

Table 4.2 Some reported molecular alterations in diffuse malignant mesothelioma

Function	Gene target	Alteration
Autocrine growth stimulation	Growth factors and receptors	HGF/MET upregulation EGFR upregulation PDGF upregulation IGF-1 upregulation
Tumour-suppressor genes	*p15, p16^{INK4A}, p14^{ARF}*	Inactivation or deletion
	Neurofibromin 2	*NF2* deletions, mutations
	RASSF1A, GPC3	Hypermethylation
Angiogenesis	VEGF	Upregulation
Apoptosis	*AKT*	Constitutive activation
	BCL-X	Upregulation

EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; PDGF, platelet-derived growth factor; RASSF1A, Ras-association domain family 1; VEGF, vascular endothelial growth factor

From Murthy & Testa (1999), Altomare *et al.* (2005), Catalano *et al.* (2005), Kratzke & Gazdar (2005), Cacciotti *et al.* (2006), NIOSH (2009)

regulatory genes as well as inflammation-associated genes and apoptosis-related genes (Tsou *et al.*, 2007; Christensen *et al.*, 2008). Christensen *et al.* (2009) hypothesized that hypermethylation of specific genes confers a selective survival advantage to preneoplastic mesothelial cells in a microenvironment of persistent tissue injury and/or oxidative stress associated with exposure to asbestos fibres.

In summary, these new genomic and proteomics approaches offer promise for the discovery of novel biomarkers associated with the development of diffuse malignant mesothelioma following exposure to asbestos or erionite. No specific marker is yet available to identify those cancers.

4.3 Mechanisms of carcinogenesis

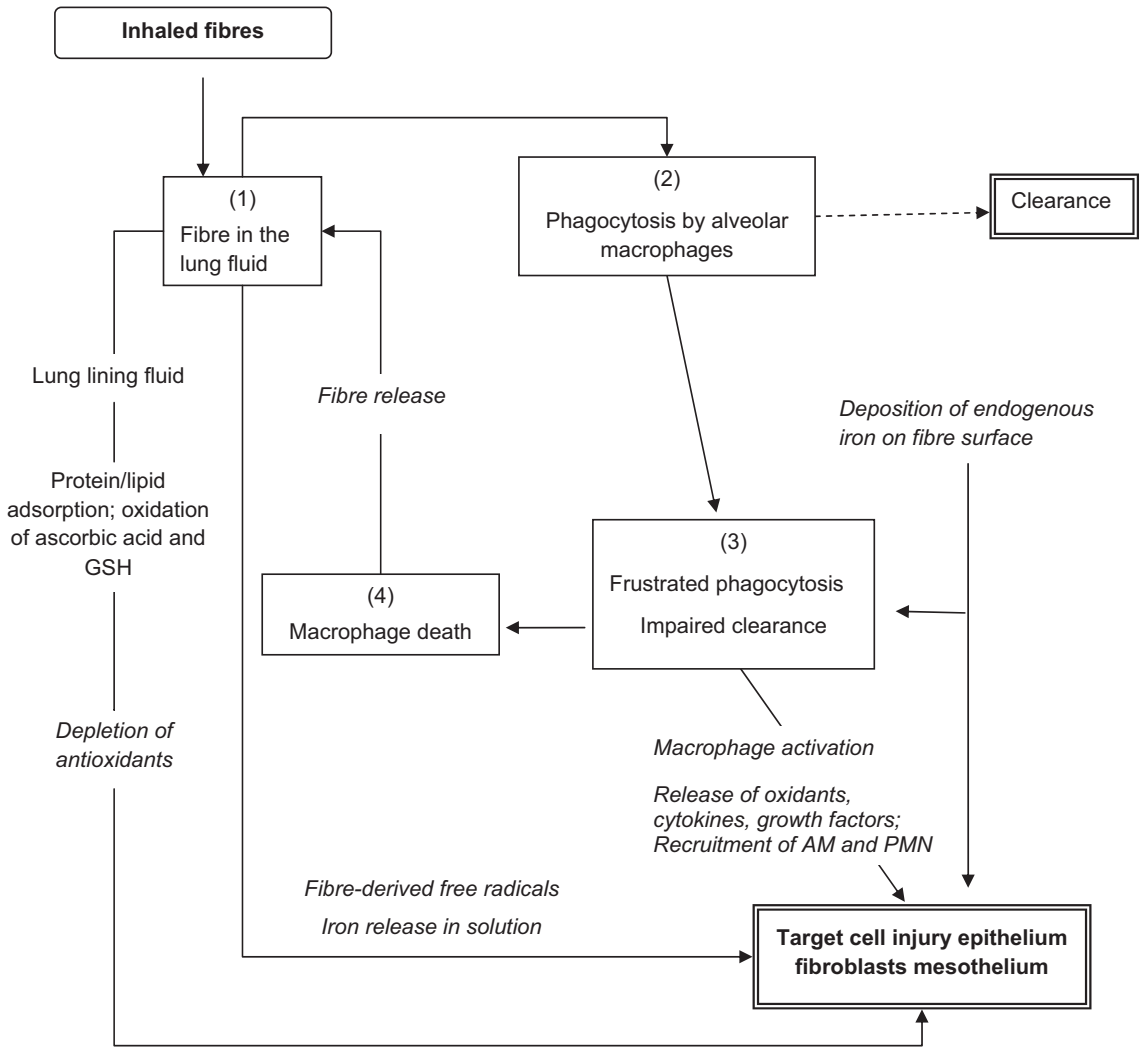
4.3.1 Physicochemical properties of mineral fibres associated with toxicity

Asbestos are natural fibrous silicates, with similar chemical composition (silica framework includes various metal cations, typically Mg^{2+}, Ca^{2+}, $Fe^{2+/3+}$, Na^{+}) mostly differing in the crystallographic constraints that yield the fibrous habit. They are poorly soluble minerals which only undergo selective leaching and incongruent dissolution. Erionite is a zeolite, which often crystallizes in thin long fibres. Major determinants of toxicity are form and size of the fibres, surface chemistry, and biopersistence. Crystal structure, chemical composition, origin, and associated minerals, as well as trace contaminants, modulate surface chemistry; and transformation, translocation, and solubility of the fibres in body fluids influence their biopersistence, a factor which modulates cumulative exposure (Fubini, 1997; Bernstein *et al.*, 2005; Fubini & Fenoglio, 2007; Sanchez *et al.*, 2009; Fig. 4.1).

(a) Crystal structure

Asbestos minerals can be divided into two groups: serpentine asbestos (chrysotile [$Mg_3Si_2O_5(OH)_4$]), and amphibole asbestos (crocidolite [$Na_2(Mg,Fe^{2+})_3Fe_2^{3+}Si_8O_{22}(OH)_2$], amosite [$(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2$], tremolite [$Ca_2Mg_5Si_8O_{22}(OH)_2$], actinolite [$Ca_2(Mg,Fe^{2+})_5Si_8O_{22}(OH)_2$], and anthophyllite [$Mg_7Si_8O_{22}(OH)_2$]). Formulae reported are ideal and are always significantly modified in nature by the occurrence of several substituting cations (e.g. $Fe^{2+/3+}$, Al^{3+}, Na^{+}). The crystal structure of chrysotile results from the association of a tetrahedral silicate sheet of composition $(Si_2O_5)_n^{2n-}$ with an octahedral brucite-like sheet of composition $[Mg_3O_2(OH)_4]_n^{2n+}$, in which iron substitutes for magnesium. The two sheets are bonded to form a 1:1 layer silicate; a slight misfit between the sheets causes curling to form

Fig. 4.1 Physicochemical properties involved in the biological activity of asbestos fibres



AMs, alveolar macrophages; GSH, glutathione; PMNs, polymorphonuclear neutrophils
Adapted from Fubini & Otero Areán (1999), Fubini & Fenoglio (2007)

concentric cylinders, with the brucite-like layer on the outside. Van der Waals interparticle forces hold together fibrils into the actual fibre so that, when chrysotile breaks up, a large number of smaller fibres or fibrils are generated (Fubini & Otero Areán, 1999).

Amphiboles have an intrinsically elongated crystal structure which breaks up along planes within the crystal structure itself into progressively smaller fragments that generally retain a fibrous aspect. This structure can be described in terms of a basic structural unit formed by a double tetrahedral chain (corner-linked SiO_4 tetrahedra) of composition $(Si_4O_{11})_n^{6n-}$. These silicate double-chains share oxygen atoms with alternate layers of edge-sharing MO_6 octahedra, where M stands for a variety of cations: mostly Na^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, or Fe^{3+} (Fubini & Otero Areán, 1999).

(b) Form and size

The pathogenic potential of asbestos depends upon its aspect ratio and fibre size. Fibre size affects respirability (respiratory zone falls off above aerodynamic diameters of 5 µm) and clearance by alveolar macrophages (section 4.1.1) (Donaldson & Tran, 2004). Short fibres are cleared more efficiently than longer ones, which undergo frustrated phagocytosis by macrophages. Short amosite fibres obtained by grinding long ones are less inflammogenic (Donaldson *et al.*, 1992), induce fewer chromosomal aberrations (Donaldson & Golyasnya, 1995), and reduce the inhibition of the pentose phosphate pathway (Riganti *et al.*, 2003). In-vitro genotoxicity studies demonstrated that both short and intermediate chrysotile asbestos fibres induced micronuclei formation and sister chromatid exchange in Chinese hamster lung cells. Intermediate fibres were more active than short fibres even when followed by treatment with dipalmitoyl lecithin, a principal constituent of pulmonary surfactant (Lu *et al.*, 1994). Long fibres but not short fibres of amosite asbestos, opsonized with rat immunoglobin, were shown to induce a dramatic enhancement of superoxide anions in macrophages isolated from rat lung (Hill *et al.*, 1995). Asbestos bodies are formed mostly on fibres longer than 20 µm (Roggli, 2004).

The role of the aspect ratio and size appears to be different for the three major asbestos-related diseases: i) asbestosis was reported as most closely associated with the surface area of retained fibres (NIOSH, 2009) although fibrosis also correlates with fibres > 2 µm long (Dodson *et al.*, 2003); ii) mesothelioma is better related to the numbers of fibres longer than about 5 µm and thinner than about 0.1 µm; and iii) lung cancer with fibres longer than about 10 µm and thicker than about 0.15 µm (NIOSH, 2009). Several studies, however, report the presence of very short fibres in lung and pleural tissue from patients with malignant mesothelioma (Dodson *et al.*, 2003; Dodson *et al.*, 2005; Suzuki *et al.*, 2005; Dodson *et al.*, 2007), suggesting caution to exclude short fibres (< 5 µm) in the development of asbestos-related diseases (Dodson *et al.*, 2003).

(c) Surface reactivity

In the last few decades, it has been accepted that, in addition to fibrous habit, surface reactivity also plays a role in the pathogenic effects of amphibole and chrysotile asbestos. The potential to release free radicals, among various other features, is considered the major determinant of the pathogenic response.

(i) Free-radical generation

Three different mechanisms of free-radical generation may take place at the surface of asbestos fibres, each one triggered by a different kind of active surface site: i) Fenton chemistry (yielding with H_2O_2 the generation of highly reactive hydroxyl radicals HO•); ii) Haber–Weiss cycle (in the absence of H_2O_2 and Fe(II), endogenous reductants allow progressive reduction of atmospheric oxygen to HO•); iii) homolytic

rupture of a carbon-hydrogen bond in biomolecules, with generation of carbon-centred radicals in the target molecule (peptides, proteins, etc.) (Hardy & Aust, 1995; Fubini & Otero Areán, 1999; Kamp & Weitzman, 1999).

Mechanism i) is relevant only in cellular compartments where H_2O_2 is present (i.e. phagolysosomal environment in macrophages), while Mechanisms ii) and iii)_may occur ubiquitously once fibres are inhaled. All mechanisms require the presence of iron ions. One stoichiometric chrysotile prepared by chemical synthesis, thus fully iron-free, was not active in free-radical generation (cell-free tests), did not induce lipid peroxidation, nor inhibit the pentose phosphate pathway in human lung epithelial cells, which is the opposite to what is found in natural specimens (Gazzano *et al.*, 2005). When loaded with less than 1 wt.% of Fe^{3+} the synthetic chrysotile also became active (Gazzano *et al.*, 2007). Asbestos fibres deprived of iron (following treatments with chelators) do not generate hydroxyl radicals (Fubini *et al.*, 1995) or damage DNA, and are less potent in causing lipid peroxidation *in vitro* (Hardy & Aust, 1995). However, not all iron ions are equally reactive in free-radical generation, depending upon their coordination and oxidation state (Shukla *et al.*, 2003; Bernstein *et al.*, 2005). Fe (II) is active even in trace amounts (Fubini *et al.*, 1995). Furthermore, Mechanism 3 requires isolated and poorly coordinated iron ions (Martra *et al.*, 2003; Turci *et al.*, 2007). The surface sites involved in this reaction are oxidized and become inactive following thermal treatments: amphibole asbestos fibres heated up to 400°C in air (Tomatis *et al.*, 2002) lose their potential in generating carboxyl radicals, but retain the reactivity for hydroxyl radicals, most likely through Mechanism 2, as long as their crystal structure is preserved. Conversely, the reduction of ferric into ferrous ions increases the radical activity (Gulumian *et al.*, 1993a). The radical yield appears unrelated to the total amount of iron (Gulumian *et al.*, 1993b), because chrysotile shows a similar behaviour to crocidolite in cell-free tests despite the lower content of iron (3–6% versus 27%). Iron oxides (magnetite, haematite) are unable to produce radical species, whereas model solids, e.g zeolites enriched with small amount of iron but with ions poorly coordinated and mostly in low valence state, are very reactive, particularly in hydrogen abstraction (Fubini *et al.*, 1995).

Iron-derived free radicals are believed to produce a variety of cell effects including lipid peroxidation (Ghio *et al.*, 1998; Gulumian, 1999), DNA oxidation (Aust & Eveleigh, 1999), TNF-release and cell apoptosis (Upadhyay & Kamp, 2003), adhesion (Churg *et al.*, 1998), and an increase of fibre uptake by epithelial cells (Hobson *et al.*, 1990).

(ii) Iron bioavailability and biodeposition

Iron can be removed from asbestos fibres by intracellular chelators. If iron is mobilized from low–molecular-weight chelators, e.g. citrate, redox activity may be altered. The chelator–iron complex can diffuse throughout the cell, and catalyse the formation of hydroxyl radicals. Mobilization of iron was shown to correlate with DNA strand breaks and with DNA oxidation induced by crocidolite, amosite, and chrysotile (Hardy & Aust, 1995). In human lung epithelial and pleural mesothelial cells, the extent of iron mobilization was also related to the inactivation of epidermal growth factor receptor (EGFR/ErbB1), a step in the pathway leading to apoptosis (Baldys & Aust, 2005).

Mineral fibres may also acquire iron which, under certain conditions, may modify their reactivity. Erionite (Dogan *et al.*, 2008) is able to bind both ferrous (through ion exchange) and ferric ions (through a precipitation or crystallization process). After ferrous-binding, erionite acquires the ability to generate hydroxyl radicals, and to catalyse DNA damage (DNA single-strand breaks); and after ferric-binding, the reactivity is acquired only in the presence of a reductant

(Hardy & Aust, 1995; Fach *et al.*, 2003; Ruda & Dutta, 2005). During their residence in the lung, asbestos fibres, like erionite fibres, acquire iron via a complex mechanism that may originate from the adsorption and disruption of ferritin, eventually yielding ferruginous bodies. These so-called asbestos bodies are preferentially formed onto long amphibole fibres but have also been found onto chrysotile fibres (Roggli, 2004). Although the presence of asbestos bodies in asbestos-related diseases is well documented, their biological role is still controversial. Iron deposition was thought to protect cells (Ghio *et al.*, 1997), but, deposited iron may become redox-active, thus enhancing the catalytic potential of the fibres (Ghio *et al.*, 2004). Asbestos bodies with amosite cores caused DNA single-strand breaks (Lund *et al.*, 1994); and increased radical damage to DNA was reported for ferritin-covered amosite in the presence of ascorbic acid (Otero-Areán *et al.*, 1999). Asbestos fibres might also disrupt normal iron homeostasis in the host by mobilizing and accumulating this metal (Ghio *et al.*, 2008).

Binding Fe (II) from solution increases iron mobilization from crocidolite by chelators, and induces DNA single-strand breaks. Increased lipid peroxidation and release of leukotriene B4 is found in alveolar macrophages from rats treated with Fe (III)-loaded crocidolite, and Fe (III)-loaded crocidolite fibres induce more DNA single-strand breaks *in vitro* than do untreated crocidolite fibres (Ghio *et al.*, 1992).

It was suggested that crocidolite stimulates inductible nitric oxide synthase by decreasing iron bioavailability (Aldieri *et al.*, 2001).

(d) Biopersistence, biodurability, and ecopersistence

The residence time in the lung depends upon both the clearance mechanisms and physico-chemical processes taking place. Clearance mechanisms are mainly related to the shape and size of the particle, whereas chemical composition, surface area, and structural parameters mainly affect leaching, dissolution, and breakage.

Selective leaching is more pronounced for serpentine asbestos than for amphiboles, which have no leachable "weak points" in their structure. Selective leaching of chrysotile occurs under strong acidic or chelating conditions, resulting in removal of Mg^{2+} ions. The kinetics vary according to the origin of the material, mechanical treatments, and associated contaminants, e.g. presence of nemalite (fibrous brucite) (Morgan, 1997). Chrysotile may lose magnesium *in vivo*, following phagocytosis by alveolar macrophages. The biological potential of magnesium-depleted chrysotile is greatly decreased (Langer & Nolan, 1994; Gulumian, 2005). Furthermore, leached fibres undergo breakage into shorter fibres, which may be cleared more readily from the lung. This accounts for the relatively low biopersistence of chrysotile compared to the amphiboles. The lungs of some chrysotile workers at autopsy contain low levels of chrysotile but substantial numbers of tremolite fibres, which is present in some chrysotile-bearing ores. For this reason, tremolite has been suggested to contribute to the carcinogenic effects seen in chrysotile miners (McDonald *et al.*, 1997; McDonald & McDonald, 1997; McDonald, 1998). Other asbestiform minerals may be associated with chrysotile, and, in some cases, modulate its toxicity, depending upon their amount and physicochemical characteristics. Balangeroite, occasionally intergrows with chrysotile (up to 5%) in the Balangero mine (Italy) and its sourroundings. Balangeroite fibres have a different structure from amphiboles, and are poorly eco- and bio-durable (Favero-Longo *et al.*, 2009; Turci *et al.*, 2009). Balangeroite may contribute to the overall toxicity of chrysotile, but it cannot be compared to tremolite nor considered to be solely responsible for the excess of mesothelioma found in Balangero (Mirabelli *et al.*, 2008).

In the natural environment, weathering processes carried out by micro-organisms

may induce chrysotile-leaching, contributing to its bioattenuation (Favero-Longo *et al.*, 2005). However, the dissolution of chrysotile is very low, because any breakdown of the silica framework takes place at a slow rate (Hume & Rimstidt, 1992), and is limited to a few layers in mild conditions (Gronow, 1987). Even in a strong acidic environment, the final product still retains a fibrous aspect at the nanoscale which is devoid of cations (Wypych *et al.*, 2005).

4.3.2 Direct genotoxicity

Mineral fibres may directly induce genotoxicity by catalysing the generation of reactive oxygen species resulting in oxidized DNA bases and DNA strand breaks that can produce gene mutations if not adequately repaired (IOM, 2006). Both asbestos and erionite fibres can induce DNA damage mediated by reactive oxygen species. Asbestos fibres have also been shown to physically interfere with the mitotic apparatus, which may result in aneuploidy or polyploidy, and specific chromosomal alterations characteristic of asbestos-related cancer (Jaurand, 1996).

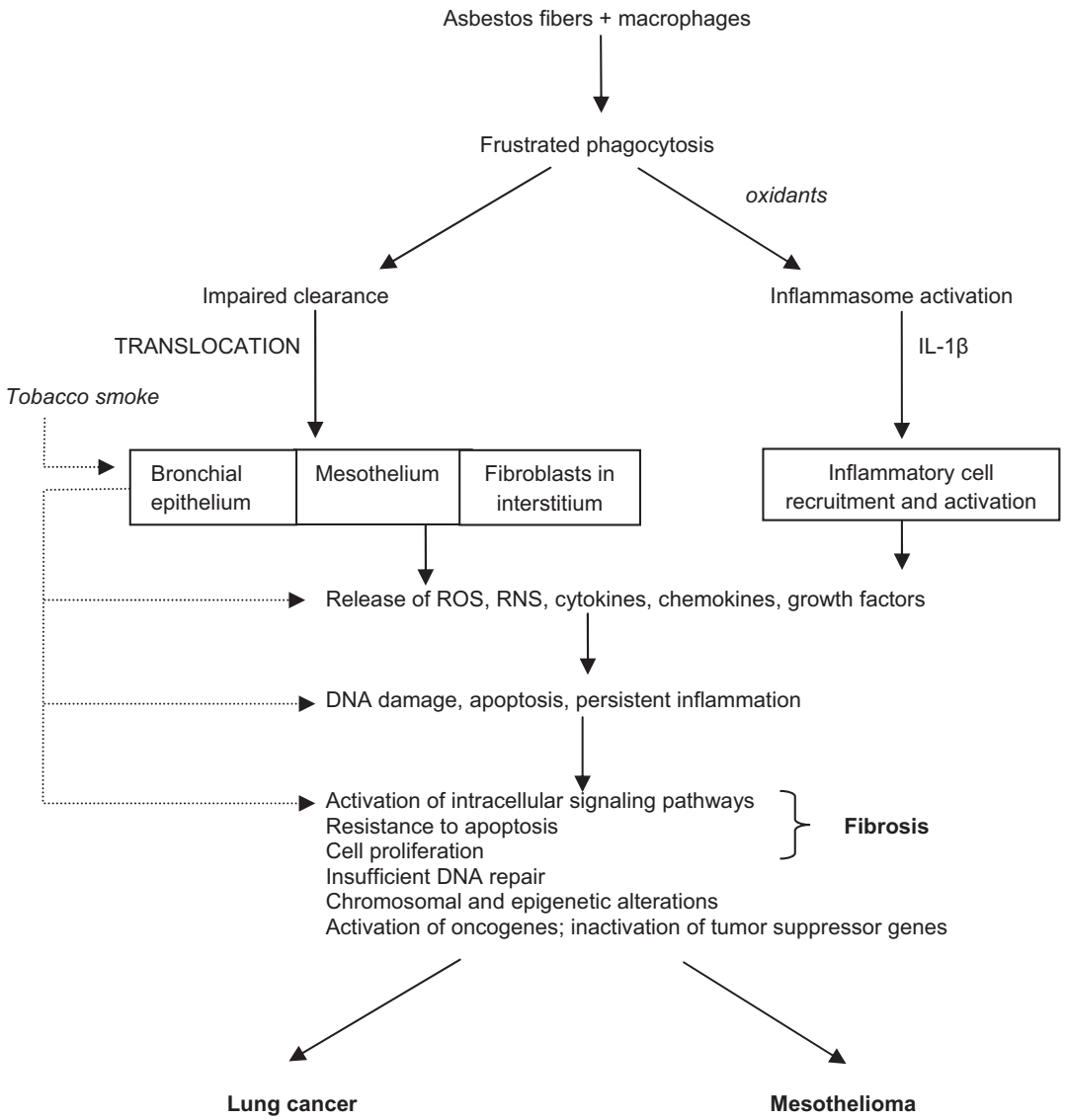
In addition to direct clastogenic and aneuploidogenic activities that may be induced following the translocation of asbestos fibres to target cell populations in the lungs, persistent inflammation and macrophage activation can secondarily generate additional reactive oxygen species, and reactive nitrogen species that can indirectly induce genotoxicity in addition to activation of intracellular signalling pathways, stimulation of cell proliferation and survival, and induction of epigenetic alterations (Fig. 4.2).

4.3.3 Indirect mechanisms

Asbestos fibres have unique and potent effects on alveolar macrophages that have been postulated to trigger the chain of events leading to chronic lung fibrosis (asbestosis), and lung cancer (Shukla *et al.*, 2003). Macrophages express a variety of cell-surface receptors that bind to mineral fibres leading to phagocytosis, macrophage apoptosis, or macrophage activation. Receptors expressed by macrophages and other target cells in the lung that bind mineral fibres include MARCO, a scavenger receptor class A, and integrin receptors (Boylan *et al.*, 1995; Gordon *et al.*, 2002; Arredouani *et al.*, 2005). Macrophage apoptosis has also been postulated to contribute to an increased incidence of autoimmune diseases in residents in Libby, Montana, USA, who are exposed to vermiculite contaminated with amphibole asbestos fibres (Noonan *et al.*, 2006; Blake *et al.*, 2008).

Phagocytosis of asbestos fibres leads to the excess generation of reactive oxygen and nitrogen species by both direct (described in Sections 4.3.1 and 4.3.2), and indirect mechanisms (Manning *et al.*, 2002). Alveolar macrophages phagocytize particulate materials and micro-organisms leading to assembly of NADPH oxidase in the phagolysosomal membrane that generates reactive oxygen species, which are potent antimicrobial agents. Asbestos fibres have elevated surface reactivity and redox-active iron that can generate hydroxyl radicals leading to lipid peroxidation, protein oxidation, and DNA damage resulting in lung injury that is amplified by persistent inflammation (Fig. 4.1 and 4.2). Recent investigations in genetically engineered mice have provided evidence for a key role of the NALP3 inflammasome as an intracellular sensor of the initial interactions between asbestos fibres and other crystals such as monosodium urate with macrophages (Yu & Finlay, 2008). The NALP3 inflammasome activates caspase-1 that cleaves IL-1β precursor to active IL-1β that is rapidly secreted (Cassel *et al.*, 2008; Dostert *et al.*, 2008). This cytokine then triggers the recruitment and activation of additional inflammatory cells and the release of additional cytokines including TNF-α, IL-6, and IL-8 that perpetuate a prolonged inflammatory response to these biopersistent mineral dusts (Shukla *et al.*, 2003).

Fig. 4.2 Proposed mechanism for the carcinogenicity of asbestos fibres



IL-1β, interleukin -1β; RNS, reactive nitrogen species; ROS, reactive oxygen species.
Adapted from Shukla *et al.* (2003), Kane (2006), Nymark *et al.* (2008)

The generation of reactive oxygen species by asbestos fibres has also been associated with inducing apoptosis in mesothelial cells (Broaddus *et al.*, 1996), and alveolar epithelial cells (Aljandali *et al.*, 2001).

Asbestos fibres have been shown to contribute to the transformation of a variety of target cells from different species *in vitro*, and to induce lung tumours and malignant pleural mesothelioma in rodents following chronic inhalation (Bernstein *et al.*, 2005). There are important species differences in the induction of asbestos-related cancers: rats are more susceptible to the induction of lung cancer, and hamsters are resistant to the induction of lung cancer but more susceptible to the development of malignant pleural mesothelioma (IARC, 2002). Subchronic inhalation studies using refractory ceramic fibres (RCF-1) suggest that the increased susceptibility of hamsters to developing malignant pleural mesothelioma may be related to greater translocation and accumulation of fibres in the pleural space, and an increased mesothelial cell proliferation in hamsters compared to rats (Gelzleichter *et al.*, 1999). There are serious limitations in extrapolating these species differences to humans. First, most human lung cancers, even in asbestos-exposed individuals, are confounded by tobacco smoke that has potent independent genotoxic effects as reviewed later in Section 4.4.1. Second, diffuse malignant mesothelioma in humans is usually diagnosed at an advanced stage, and there are no reliable premalignant changes or biomarkers that may provide clues about the molecular pathogenesis of mesothelioma associated with exposure to asbestos or erionite fibres (NIOSH, 2009).

A unifying mechanism based on the experimental in-vitro cellular and in-vivo rodent models is proposed in Fig. 4.2.

Recent biochemical studies have confirmed that oxidative damage to cytosine is a plausible biological mechanism leading to epigenetic alterations and development of cancer in association with persistent inflammation (Valinluck & Sowers, 2007). Neutrophils and macrophages are the source of reactive oxygen and nitrogen species triggered by phagocytosis of crystalline silica (quartz) or asbestos fibres. In addition, myeloperoxidase catalyses the formation of hypochlorous acid (HOC1) in neutrophils, and a specific peroxidase catalyses the formation of hypobromous acid (HOBr) in eosinophils (Babior, 2000). The formation of 8-oxoguanine, 5-hydroxymethylcytosine, or 5-hydroxycytosine interferes with DNA methylation and binding of methyl-CpG binding domains (MBDs). In contrast, chlorination or bromination of cytosine mimics 5-methylcytosine and induces heritable DNA methylation at previously unmethylated sites. Halogenated cytosines are also recognized by MBDs to facilitate chromatin remodelling. However, these modified bases are not recognized by DNA glycosylase, and are not repaired (Valinluck & Sowers, 2007).

This hypothesis linking heritable alterations in patterns of cytosine methylation with endogenous sources of oxidants released from inflammatory cells is a plausible explanation for the development of lung cancer and diffuse malignant mesothelioma associated with exposure to mineral fibres. Elevated neutrophils and eosinophils have been found in the pleural space following the inhalation of refractory ceramic fibres by hamsters and rats (Gelzleichter *et al.*, 1999). Furthermore, myeloperoxidase activity has been detected in rodent lungs following exposure to asbestos fibres, whereas a decreased lung inflammation was observed in asbestos-exposed myeloperoxidase-null mice (Haegens *et al.*, 2005). This indirect mechanism secondary to persistent inflammation may be responsible for altered epigenetic methylation profiles, which are characteristic of human malignant pleural mesotheliomas (Christensen *et al.*, 2009).

4.4 Susceptible populations

Both exogenous environmental and occupational exposures and endogenous factors including genetic susceptibility contribute to the development of lung cancer (NIOSH, 2009) and diffuse malignant mesothelioma (Weiner & Neragi-Miandoab, 2009). The best example of an exogenous exposure that is a major cofactor with asbestos fibres in the development of cancer of the larynx and of the lung is tobacco smoking (Table 4.3; Table 4.4; IARC, 2004; IOM, 2006). Additional environmental and occupational exposures are also risk factors for cancer of the larynx (Table 4.3) and of the lung (Table 4.4); these exposures are potential confounders in human epidemiological studies (IOM, 2006). Specific examples of these cofactors and other environmental and occupational exposures will be described in relationship to mechanisms of these cancers associated with mineral dust exposures.

4.4.1 Other risk factors for cancer of the lung and of the larynx, and diffuse malignant mesothelioma

(a) Tobacco smoke

Co-exposure to tobacco smoke and asbestos fibres is at least additive and possibly multiplicative in the development of lung cancer (Vainio & Boffetta, 1994). The inhalation of tobacco smoke (Walser *et al.*, 2008) as well as mineral fibres is associated with excess generation of reactive oxygen and nitrogen metabolites, cell injury and apoptosis, and persistent lung inflammation (Shukla *et al.*, 2003; IARC, 2004). Excess oxidant generation has been shown to enhance the penetration of asbestos fibres into respiratory epithelial cells, and to impair fibre clearance (McFadden *et al.*, 1986; Churg *et al.*, 1989), as well as altering the metabolism and detoxification of tobacco smoke carcinogens (Nymark *et al.*, 2008). Asbestos fibres can also adsorb tobacco smoke carcinogens and metals and facilitate their transport into the lungs (IOM, 2006). Asbestos fibres have also been shown to activate growth-factor receptors and cell-signalling pathways that stimulate cell proliferation and promote cell survival (Albrecht *et al.*, 2004). In summary, co-exposures to tobacco smoke and mineral fibres can amplify acquired genetic mutations induced by tobacco smoke carcinogens, and amplify cell proliferation in response to tissue injury leading to an increased risk for the development of cancer of the larynx and of the lung (Nymark *et al.*, 2008).

Table 4.3 Risk factors for the development of cancer of the larynx

Exposure	Reference
Active tobacco smoking	IARC (1986, 2004, 2012d)
Alcohol	IARC (1988, 2010, 2012d)
Mustard gas	IARC (1987a, 2012e)
Inorganic acid mists containing sulfuric acid	IARC (1992, 2012e)
Asbestos fibres	IOM (2006), IARC (2012b)
Human papilloma virus (HPV): types 6, 11, 16, 18 limited evidence	IARC (2007, 2012c)

Compiled by the Working Group

(b) Other occupational and environmental exposures

Alcohol and occupational exposure to irritants (Table 4.3) also contribute to the development of cancer of the larynx. These irritants, similar to inhalation of tobacco smoke, can cause repeated episodes of injury to the respiratory epithelium, resulting in metaplasia and dysplasia (Olshan, 2006); these preneoplastic lesions may then acquire additional molecular alterations and progress towards the development of invasive lung or laryngeal carcinoma. Other occupational exposures responsible for the development of lung cancer include direct-acting carcinogens such as ionizing radiation (IARC, 2000, 2012a), and metals (reviewed in IARC, 2012b).

Table 4.4 Risk factors for the development of cancer of the lung

Exposure	Reference
Active and passive tobacco smoking	IARC (2004, 2012d)
Ionizing radiation	IARC (2000, 2012a)
Respirable dusts and fibres:	
Asbestos	IARC (1987a, 2012b)
Talc containing asbestiform fibres	IARC (1987a, 2012b)
Erionite	IARC (1987a, 2012b)
Crystalline silica (quartz)	IARC (1997, 2012b)
Vermiculite contaminated with asbestos fibres	Amandus & Wheeler (1987), McDonald *et al.* (2004), IARC (2012b)
Bis(chloromethyl)ether and chloromethyl methyl ether	IARC (1987a, 2012e)
Arsenic and arsenic compounds	IARC (1987a, 2012b)
Beryllium	IARC (1993, 2012b)
Cadmium and cadmium compounds	IARC (1993, 2012b)
Hexavalent chromium	IARC (1990, 2012b)
Nickel sulfate, oxides, and sulfides	IARC (1990, 2012b)
Soots	IARC (1985, 1987a, 2012e)

Compiled by the Working Group

The strongest risk factors associated with the development of diffuse malignant mesothelioma include environmental or occupational exposures to erionite, asbestos fibres, and talc or vermiculite contaminated with asbestos fibres (Table 4.5; NIOSH, 2009). It is unknown whether the carcinogenic effects of exposure to mixed dusts contaminated with asbestos fibres can be entirely attributed to the asbestos fibres or whether co-exposure to talc or vermiculite dusts potentiates the retention and/or biological activity of asbestos fibres *in vivo* (Davis, 1996). The occurrence of talc pneumoconiosis and its relationship to other mineral dust contaminants including quartz and tremolite was recently reviewed (IARC, 2010). In-vitro assays of talc cytotoxicity were also summarized (IARC, 2010). No experimental studies have been published assessing the cytotoxicity of vermiculite contaminated with asbestos fibres. A sample of the mixture of amphibole fibres associated with Libby vermiculite ore has been shown to induce cytotoxicity and oxidative stress in macrophages *in vitro* (Blake *et al.*, 2007).

(c) SV40 and HPV viruses

Two human DNA tumour viruses have been linked with an increased risk for cancer of the larynx (Table 4.3; high-risk subtypes of human papillomavirus (HPV)) and diffuse malignant mesothelioma (Table 4.5; Simian virus 40 (SV40)).

The evidence for HPV 16 in the development of cancer of the larynx has been evaluated as limited, although it has been implicated as an independent risk factor in the development of other squamous cell carcinomas arising in the head and neck region (IARC, 2007, 2012c).

The association between exposure to SV40 and asbestos fibres in the development of diffuse malignant mesothelioma is highly controversial (Butel & Lednicky, 1999; Gazdar *et al.*, 2002; Shah, 2004; IOM, 2006). SV40 is not an essential cofactor for the development of mesothelioma; for example, residents of the Cappadocian villages in Turkey have a very high risk for diffuse malignant mesothelioma but do not have evidence of SV40 exposure (Dogan *et al.*, 2006). Although there are several in-vitro mechanistic

Table 4.5 Risk factors for the development of diffuse malignant mesothelioma

Exposure	Reference
Asbestos fibres	IARC (1987a, 2012b)
Erionite	IARC (1987a, 2012b)
Talc containing asbestiform fibres	IARC (1987a, 2012b)
Vermiculite contaminated with asbestos fibres	Amandus & Wheeler (1987), IARC (1987a, 2012e), McDonald *et al.* (2004)
Thorotrast	IARC (2001, 2012a)

Compiled by the Working Group

studies that support a role for SV40 viral oncogenes in the transformation of mesothelial cells, the human epidemiological evidence is inconclusive to support a causal association (Weiner & Neragi-Miandoab, 2009).

4.4.2 Genetic susceptibility

(a) Cancer of the lung

Tobacco smoke is the major cause of cancer of the lung; however, only a few rare hereditary syndromes are associated with an increased risk of lung, as well as other cancers: Bloom syndrome, Li-Fraumeni syndrome, and hereditary retinoblastoma (Lindor *et al.*, 2006). Other genetic polymorphisms in genes related to the metabolism and detoxification of tobacco smoke carcinogens, antioxidant defenses, and DNA repair have been suggested as predisposing factors for the development of lung cancer, although individually they contribute minimally to an increased risk (IOM, 2006). Attempts have been made to identify genetic polymorphisms in enzymes involved in xenobiotic metabolism and antioxidant defense that increase the risk for asbestos-related lung cancer; however, no consistent associations have been found (Nymark *et al.*, 2008).

(b) Diffuse malignant mesothelioma

With the exception of certain populations who have been exposed environmentally to asbestos or erionite fibres since birth (NIOSH, 2009), the development of diffuse malignant mesothelioma even in occupationally exposed workers is less common than the development of lung cancer (Nymark *et al.*, 2008). This observation has led to the hypothesis that there may be a genetic predisposition to the development of diffuse malignant mesothelioma following exposure to asbestos or erionite fibres. Isolated case reports provide examples of diffuse malignant mesothelioma in patients with neurofibromatosis type 2 (Baser *et al.*, 2002) or Li-Fraumeni syndrome (Heineman *et al.*, 1996) who are also exposed to asbestos. Several reports of familial cases of diffuse malignant mesothelioma are complicated by a common household exposure history (Weiner & Neragi-Miandoab, 2009). The strongest association between environmental exposure to erionite and genetic susceptibility to diffuse malignant mesothelioma has been provided by pedigree analysis of residents in the Cappadocia region of Turkey (Dogan *et al.*, 2006). However, there is skepticism about the accuracy of this analysis, and a recent review indicated that familial clusters can account for only 1.4% of cases of mesothelioma in Italy between 1978–2005 (Ascoli *et al.*, 2007; Ugolini *et al.*, 2008). One study has reported an association between genetic polymorphisms in the X-ray complementing group 1 gene (XRCC1) and the development of malignant mesothelioma in a population exposed to asbestos fibres (Dianzani *et al.*, 2006). More sensitive genome-wide association studies may uncover new markers for genetic susceptibility that predict increase risks of developing diffuse malignant mesothelioma following exposure to asbestos or erionite fibres.

4.5 Synthesis

The mechanistic basis for asbestos carcinogenicity is a complex interaction between crystalline mineral fibres and target cells *in vivo*. The most important physicochemical properties of asbestos fibres related to pathogenicity are surface chemistry and reactivity, surface area, fibre dimensions, and biopersistence. Multiple direct and indirect mechanisms have been proposed based on numerous in-vitro cellular assays, and acute and subchronic animal bioassays. These complex mechanisms most likely interact at multiple stages during the development of lung cancer and diffuse malignant mesothelioma.

The following general mechanisms have been proposed for the carcinogenicity of asbestos fibres (Fig. 4.1; Fig. 4.2):

1. Direct interaction between asbestos fibres and target cells *in vitro*:
   - Asbestos and erionite fibres have been shown to generate free radicals that directly induce genotoxicity as assessed by DNA breaks and oxidized bases in DNA.
   - Asbestos fibres have also been shown to interfere with the mitotic apparatus by direct physical interaction resulting in aneuploidy and polyploidy.
2. Indirect mechanisms:
   - In laboratory animals, asbestos fibres have been shown to induce macrophage activation and persistent inflammation that generate reactive oxygen and nitrogen species contributing to tissue injury, genotoxicity, and epigenetic alterations. Persistent inflammation and chronic oxidative stress have been associated with the activation of intracellular signalling pathways, resistance to apoptosis, and stimulation of cell proliferation.

There are significant species differences in the responses of the respiratory tract to the inhalation of asbestos fibres. The biological mechanisms responsible for these species differences are unknown. Based on comparative animal experimental studies, there may be differences in deposition and clearance of fibres in the lungs, in severity of fibrosis, in kinetics of translocation of fibres to the pleura, and in levels or types of antioxidant defense mechanisms.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite, and anthophyllite). Asbestos causes mesothelioma and cancer of the lung, larynx, and ovary. Also positive associations have been observed between exposure to all forms of asbestos and cancer of the pharynx, stomach, and colorectum. For cancer of the colorectum, the Working Group was evenly divided as to whether the evidence was strong enough to warrant classification as *sufficient*.

There is *sufficient evidence* in experimental animals for the carcinogenicity of all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite and anthophyllite).

All forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite and anthophyllite) are *carcinogenic to humans (Group 1)*.

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SILICA DUST, CRYSTALLINE, IN THE FORM OF QUARTZ OR CRISTOBALITE

Silica was considered by previous IARC Working Groups in 1986, 1987, and 1996 (IARC, 1987a, b, 1997). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

Silica, or silicon dioxide (SiO_2), is a group IV metal oxide, which naturally occurs in both crystalline and amorphous forms (i.e. polymorphic; NTP, 2005). The various forms of crystalline silica are: α-quartz, β-quartz, α-tridymite, β-tridymite, α-cristobalite, β-cristobalite, keatite, coesite, stishovite, and moganite (NIOSH, 2002). The most abundant form of silica is α-quartz, and the term quartz is often used in place of the general term crystalline silica (NIOSH, 2002).

1.1 Identification of the agent

α-Quartz is the thermodynamically stable form of crystalline silica in ambient conditions. The overwhelming majority of natural crystalline silica exists as α-quartz. The other forms exist in a metastable state. The nomenclature used is that of α for a lower-temperature phase, and β for a higher-temperature phase. Other notations exist and the prefixes low- and high- are also used (IARC, 1997). The classification and nomenclature of silica forms are summarized in Table 1.1. For more detailed information, refer to the previous *IARC Monograph* (IARC, 1997).

1.2 Chemical and physical properties of the agent

Selected chemical and physical properties of silica and certain crystalline polymorphs are summarized in Table 1.1. For a detailed discussion of the crystalline structure and morphology of silica particulates, and corresponding physical properties and domains of thermodynamic stability, refer to the previous *IARC Monograph* (IARC, 1997).

1.3 Use of the agent

The physical and chemical properties of silica make it suitable for many uses. Most silica in commercial use is obtained from naturally occurring sources, and is categorized by end-use or industry (IARC, 1997; NTP, 2005). The three predominant commercial silica product categories are: sand and gravel, quartz crystals, and diatomites.

Table 1.1 Nomenclature, CAS numbers, and classification of silica forms with selected physical and chemical properties

Name	CAS No.	Basic Formula	Classification	Synonyms	Properties
Silica	7631-86-9	SiO_2	α-quartz, β-quartz; α-tridymite, β1-tridymite, β2-tridymite; α-cristobalite, β-cristobalite; coesite; stishovite; moganite		Structure: crystalline, amorphous, cryptocrystalline Molecular weight: 60.1 Solubility: poorly soluble in water at 20 °C and most acids; increases with temperature and pH Reactivity: reacts with alkaline aqueous solutions, with hydrofluoric acid (to produce silicon tetrafluoride gas), and catechol
Crystalline Silica					
Cristobalite	14464-46-1		α-cristobalite, β-cristobalite		
Quartz	14808-60-7		α-quartz, β-quartz	α-quartz: agate; chalcedony; chert; flint; jasper; novaculite; quartzite; sandstone; silica sand; tripoli	Solubility: 6–11 μg/cm^3 (6–11 ppm) at room temperature; slightly soluble in body fluids Thermodynamic properties: melts to a glass; coefficient of expansion by heat—lowest of any known substance
Tripoli	1317-95-9				
Tridymite	15468-32-3		α-tridymite, β1-tridymite, β2-tridymite		

From IARC (1997), NIOSH (2002), NTP (2005)

1.3.1 Sand and gravel

Although silica sand has been used for many different purposes throughout history, its most ancient and principal use has been in the manufacture of glass (e.g. containers, flat plate and window, and fibreglass). Sands are used in ceramics (e.g. pottery, brick, and tile), foundry (e.g. moulding and core, refractory), abrasive (e.g. blasting, scouring cleansers, sawing and sanding), hydraulic fracturing applications, and many other uses. Several uses require the material to be ground (e.g. scouring cleansers, some types of fibreglass, certain foundry applications). In some uses (e.g. sandblasting, abrasives), grinding also occurs during use. For a more complete list of end-uses, refer to Table 8 of the previous *IARC Monograph* (IARC, 1997).

According to the US Geological Survey, world production in 2008 was estimated to be 121 million metric tons (Dolley, 2009). The leading producers were the USA (30.4 million metric tons), Italy (13.8 million metric tons), Germany (8.2 million metric tons), the United Kingdom (5.6 million metric tons), Australia (5.3 million metric tons), France (5 million metric tons), Spain (5 million metric tons), and Japan (4.5 million metric tons).

1.3.2 Quartz crystals

Quartz has been used for several thousand years in jewellery as a gem stone (e.g. amethyst, citrine), and is used extensively in both the electronics and optical components industries. Electronic-grade quartz is used in electronic circuits, and optical-grade quartz is used in windows, and other specialized devices (e.g. lasers) (IARC, 1997).

1.3.3 Diatomites

Diatomites are used in filtration, as fillers (in paint, paper, synthetic rubber goods, laboratory absorbents, anti-caking agents, and scouring powders), and as carriers for pesticides. They impart abrasiveness to polishes, flow and colour qualities to paints, and reinforcement to paper. Other uses include: insulators, absorption agents, scourer in polishes and cleaners, catalyst supports, and packing material (IARC, 1997).

According to the US Geological Survey, world production in 2008 was estimated to be 2.2 million metric tons. The USA accounted for 35% of total world production, followed by the People's Republic of China (20%), Denmark (11%), Japan (5%), Mexico (4%), and France (3%) (Crangle, 2009).

1.4 Environmental occurrence

Keatite, coesite, stishovite, and moganite are rarely found in nature. The most commonly occurring polymorphs are quartz, cristobalite and tridymite, which are found in rocks and soil. These forms of silica can be released to the environment via both natural and anthropogenic sources (e.g. foundry processes, brick and ceramics manufacturing, silicon carbide production, burning of agricultural waste or products, or calcining of diatomaceous earth). Some of these anthropogenic activities may cause transformation of one polymorph into another (NIOSH, 2002).

1.4.1 Natural occurrence

α-Quartz is found in trace to major amounts in most rock types (e.g. igneous, sedimentary, metamorphic, argillaceous), sands, and soils. The average quartz composition of major igneous and sedimentary rocks is summarized in Table 10 of the previous *IARC Monograph* (IARC, 1997). Quartz is a major component of soils, composing 90–95% of all sand and silt fractions in a soil. It is the primary matrix mineral in the metalliferous veins of ore deposits, and can also be found in semiprecious stones, such as amethyst, citrine, smoky quartz, morion, and tiger's eye (IARC, 1997).

Crystalline tridymite and cristobalite are found in acid volcanic rocks. Cristobalite also occurs in some bentonite clays, and as traces in diatomite. Although rarely found in nature, coesite and stishovite have been found in rocks that equilibrated in short-lived high-pressure environments (e.g. meteoritic impact craters), and keatite has been found in high-altitude atmospheric dusts, which are believed to originate from volcanic sources (IARC, 1997).

For a more detailed description of the natural occurrence of crystalline silica and its polymorphs in air, water and soil, refer to the previous *IARC Monograph* (IARC, 1997).

1.5 Human exposure

1.5.1 Exposure of the general population

Inhalation of crystalline silica during the use of commercial products containing quartz is thought to be the primary route of exposure for the non-occupationally exposed (i.e. general) population. Commercial products containing quartz include: cleansers, cosmetics, art clays and glazes, pet litter, talcum powder, caulk, putty, paint, and mortar. No quantitative data on potential levels of exposurem during the use of these products were available at the time of

writing (WHO, 2000). The general population may also be exposed via ingestion of potable water containing quartz particles; however, quantitative data on concentrations of quartz in potable or other forms of drinking-water were again not available (IARC, 1997; WHO, 2000).

1.5.2 Occupational exposure

Because of the extensive natural occurrence of crystalline silica in the earth's crust and the wide uses of the materials in which it is a constituent, workers may be exposed to crystalline silica in a large variety of industries and occupations (IARC, 1997). Table 1.2 lists the main industries and activities in which workers could be exposed to crystalline silica. Included in this table are activities that involve the movement of earth (e.g. mining, farming, construction, quarrying), disturbance of silica-containing products (e.g. demolition of masonry and concrete), handling or use of sand- and other silica-containing products (e.g. foundry processes, such as casting, furnace installation and repair; abrasive blasting; production of glass, ceramics, abrasives, cement, etc.).

Estimates of the number of workers potentially exposed to respirable crystalline silica have been developed by the National Institute of Occupational Safety and Health (NIOSH) in the USA and by CAREX (CARcinogen EXposure) in Europe. Based on the National Occupational Exposure Survey (NOES), conducted during 1981–83, and the *County Business Patterns 1986*, NIOSH estimated that about 1.7 million US workers were potentially exposed to respirable crystalline silica (NIOSH, 2002). Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that more than 3.2 million workers in the then 15 Member States of the European Union during 1990–93 were considered as occupationally exposed to respirable crystalline silica above background level (Kauppinen *et al.*, 2000). Nearly 87% of these workers were employed in 'construction' (n = 2080000), 'manufacture of other non-metallic mineral products' (n = 191000), 'other mining' (n = 132000), 'manufacture of pottery, china and earthenware' (n = 96000), 'manufacture of machinery except electrical' (n = 78000), 'iron and steel basic industries' (n = 68000), 'manufacture of fabricated metal products, except machinery and equipment' (n = 68000), and 'metal ore mining' (n = 55000). The countries with the highest number of potentially exposed workers were: Germany (1 million workers), the United Kingdom (580000 workers), Spain (400000 workers), Italy (250000 workers), the Netherlands (170000 workers), France (110000 workers), and Austria (100000 workers) (Kauppinen *et al.*, 2000; Mirabelli & Kauppinen, 2005; Scarselli *et al.*, 2008).

For representative data in the main industries where quantitative exposure levels were available in the published literature and/or where major occupational health studies had been conducted, refer to the previous *IARC Monograph* (IARC, 1997). These main industries include mines and quarries, foundries and other metallurgical operations, ceramics and related industries, construction, granite, crushed stone and related industries, sandblasting of metal surfaces, agriculture, and miscellaneous other operations (IARC, 1997). Data from studies and reviews on crystalline silica exposure published since the previous *IARC Monograph* are summarized below.

(a) Levels of occupational exposure

To estimate the number of US workers potentially exposed to high levels of crystalline silica and to examine trends in exposure over time, Yassin *et al.* (2005) analysed data contained in the OSHA Integrated Management Information System (IMIS) database. After exclusion of duplicate bulk and area samples, a total of 7209 personal sample measurements collected during

Table 1.2 Main activities in which workers may be exposed to crystalline silica

Industry/activity	Specific operation/task	Source material
Agriculture	Ploughing, harvesting, use of machinery	Soil
Mining and related milling operations	Most occupations (underground, surface, mill) and mines (metal and non-metal, coal)	Ores and associated rock
Quarrying and related milling operations	Crushing stone, sand and gravel processing, monumental stone cutting and abrasive blasting, slate work, diatomite calcination	Sandstone, granite, flint, sand, gravel, slate, diatomaceous earth
Construction	Abrasive blasting of structures, buildings Highway and tunnel construction Excavation and earth-moving Masonry, concrete work, demolition	Sand, concrete Rock Soil and rock Concrete, mortar, plaster
Glass, including fibreglass	Raw material processing Refractory installation and repair	Sand, crushed quartz Refractory materials
Cement	Raw materials processing	Clay, sand, limestone, diatomaceous earth
Abrasives	Silicon carbide production Abrasive products fabrication	Sand Tripoli, sandstone
Ceramics, including bricks, tiles, sanitary ware, porcelain, pottery, refractories, vitreous enamels	Mixing, moulding, glaze or enamel spraying, finishing	Clay, shale, flint, sand, quartzite, diatomaceous earth
Iron and steel mills	Refractory preparation and furnace repair	Refractory material
Silicon and ferro-silicon	Raw materials handling	Sand
Foundries (ferrous and non-ferrous)	Casting, shaking out Abrasive blasting, fettling Furnace installation and repair	Sand Sand Refractory material
Metal products including structural metal, machinery, transportation equipment	Abrasive blasting	Sand
Shipbuilding and repair	Abrasive blasting	Sand
Rubber and plastics	Raw material handling	Fillers (tripoli, diatomaceous earth)
Paint	Raw materials handling	Fillers (tripoli, diatomaceous earth, silica flour)
Soaps and cosmetics	Abrasive soaps, scouring powders	Silica flour
Asphalt and roofing felt	Filling and granule application	Sand and aggregate, diatomaceous earth
Agricultural chemicals	Raw material crushing, handling	Phosphate ores and rock
Jewellery	Cutting, grinding, polishing, buffing	Semiprecious gems or stones, abrasives
Dental material	Sandblasting, polishing	Sand, abrasives
Automobile repair	Abrasive blasting	Sand
Boiler scaling	Coal-fired boilers	Ash and concretions

From IARC, 1997

2512 OSHA inspections during 1988–2003 were analysed. The findings suggest that geometric mean crystalline silica exposure levels declined in some high-risk construction industries during the period under study, and revealed a significant decline when compared with silica exposure levels found in a previous study by Stewart & Rice (1990). Geometric mean airborne silica exposure levels among workers in the following industries were significantly lower in 1988–2003

than in 1979–87: general contractor industry (0.057 mg/m^3 versus 0.354 mg/m^3), bridge-tunnel construction industry (0.069 mg/m^3 versus 0.383 mg/m^3), and stonework masonry industry (0.065 mg/m^3 versus 0.619 mg/m^3). Silica exposures in the grey-iron industry also declined by up to 54% for some occupations (e.g. the geometric mean for "furnace operators" in 1979–87 was 0.142 mg/m^3 versus 0.066 mg/m^3 in 1988–2003). [The Working Group noted that exposure levels may not have decreased globally.]

Table 1.3 presents the more recent studies that assessed the levels of respirable crystalline silica in a range of industries and countries. Other recent exposure studies that did not measure the respirable crystalline silica components are presented below.

(b) Mines

As part of a cohort mortality study follow-up in four tin mines in China, Chen *et al.* (2006) developed quantitative exposure estimates of silica mixed dust. Workers in the original cohort were followed up from the beginning of 1972 to the end of 1994. Cumulative exposure estimates were calculated for each worker using their mine employment records and industrial hygiene measurements of airborne total dust, particle size, and free silica content collected since the 1950s. Total dust concentrations of the main job titles exposed were found to have declined from about 10–25 mg/m^3 in the beginning of the 1950s to about 1–4 mg/m^3 in the 1980s and 1990s. The respirable fraction of total dust was estimated to be 25 ± 4%, and the respirable crystalline silica concentration was estimated to be 4.3% of the total mixed mine dust

Tse *et al.* (2007) conducted a cross-sectional study to investigate the prevalence of accelerated silicosis among 574 gold miners in Jiangxi, China. Using occupational hygiene data abstracted from government documents and bulk dust data from a study in another gold mine in the region, the estimated mean concentration of respirable silica dust were reported as 89.5 mg/m^3 (range, 70.2–108.8 mg/m^3). According to government documents, the total dust concentration in underground gold mining was in the range of 102.6–159 mg/m^3 (average, 130.8 mg/m^3), and the fraction of silica in total dust was around 75.7–76.1%. No data on the proportion of respirable dust were available.

To determine dose–response relationships between exposure to respirable dust and respiratory health outcomes, Naidoo *et al.* (2006) used historical data (n = 3645) and current measurements (n = 441) to characterize exposure to respirable coal mine dust in three South African coal mines. Jobs were classified into the following exposure zones: face (directly involved with coal extraction), underground backbye (away from the coal mining face), and work on the surface. Based on the 8-hour full-shift samples collected respectively, mean respirable dust concentrations in Mines 1, 2, and 3, were as follows: 0.91 mg/m^3 (GSD, 3.39; mean silica content, 2.3%; n = 102), 1.28 mg/m^3 (GSD, 2.11; mean silica content, 1.4%; n = 63), and 1.90 mg/m^3 (GSD, 2.23; mean silica content, 2.7%; n = 73) at the face; 0.48 mg/m^3 (GSD, 2.97; mean silica content, 1.48%; n = 30), 0.56 mg/m^3 (GSD, 3.71; mean silica content, 1.35%; n = 47), and 0.52 mg/m^3 (GSD, 4.06; mean silica content, 0.9%; n = 41) in the backbye zone; and, 0.31 mg/m^3 (GSD, 3.52; mean silica content, 0.95%; n = 8), 0.15 mg/m^3 (GSD, 3.56; n = 6), and 0.24 mg/m^3 (GSD, 7.69; mean silica content, 0.64%; n = 11) in the surface zone. Based on the historical data, overall geometric mean dust levels were 0.9 mg/m^3 (GSD, 4.9), 1.3 mg/m^3 (GSD, 3.3), and 0.5 mg/m^3 (GSD, 5.6) for Mines 1, 2, and 3, respectively.

(c) Granite-quarrying and -processing, crushed stone, and related industries

Bahrami *et al.* (2008) described the personal exposure to respirable dust and respirable quartz in stone-crushing units located in western Islamic Republic of Iran. A total of 40 personal samples

Table 1.3 Respirable crystalline silica concentrations in various industries worldwide

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m^3)	Number of samples	Comments
Mines				
Hayumbu *et al.* (2008), copper mines, the Zambia		Arithmetic mean (SD; range)		Cross-sectional dust exposure assessment; bulk and personal respirable samples; NIOSH method 0600 for gravimetric analysis of respirable dust; NIOSH method 7500 for quartz analysis of bulk and respirable samples; mean personal sampling time: 307 minutes (Mine 1) and 312 minutes (Mine 2)
	Mine 1	0.14 (0.2; 0–1.3)	101	
	Mine 2	0.06 (0.06; 0–0.3)	102	
Weeks & Rose (2006), metal and non-metal mines, USA, 1998–2002		Arithmetic mean (GM)		Mine Safety and Health Administration compliance data from 4726 mines; 8-hour full-shift personal air samples; gravimetric analysis of respirable dust; NIOSH method 7500 for silica analysis; arithmetic and geometric mean exposure calculated and classified by occupation, mine, and state
	Strip and open pit mines	0.047 (0.027)	13702	
	Mills or preparation plants	0.045 (0.027)	1145	
	Underground mines	0.050 (0.029)	1360	
	Overall	0.047 (0.027)	16207	
Bråtveit *et al.* (2003) underground small-scale mining, United Republic of Tanzania, 2001		Geometric mean (GSD)		Personal dust sampling (respirable and total dust) on 3 consecutive day shifts; sampling time varied between 5 and 8 hours; gravimetric analysis of respirable and total dust; NIOSH method 7500 for silica analysis
	Drilling, blasting, and shovelling	2.0 (1.7)	6	
	Shovelling and loading of sacks	1.0 (1.5)	3	
	Overall	1.6 (1.8)	9	
Park *et al.* (2002) diatomaceous earth mining and milling, California, USA, 1942–94	Mines and mills	Arithmetic mean 0.29 Cumulative exposure (mg/m^3–yr) 2.16	NR	Re-analysis of data from a cohort of 2342 California diatomaceous earth workers; mean concentration of respirable crystalline silica averaged over years of employment of cohort; crystalline silica content of bulk samples varied from 1–25%, and depended on process location
Mamuya *et al.* (2006) underground coal mining, United Republic of Tanzania; June–August 2003 and July–August 2004		Geometric mean (GSD)		Personal dust samples collected during two periods in 2003 and 2004; 134 respirable dust samples collected and analysed gravimetrically; 125 samples analysed for quartz using NIOSH method 7500
	Development team	0.073 (11.1)	56	
	Mine team	0.013 (2.97)	45	
	Transport team	0.006 (1.84)	11	
	Maintenance team	0.016 (11.05)	13	
	Overall	0.027 (8.18)	125	

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m³)	Number of samples	Comments
Granite-quarrying and -processing, crushed stone, and related industries				
Wickman & Middendorf (2002) Granite–quarrying, Georgia, USA; May 1993–February 1994	Granite sheds	Arithmetic mean (SD) 0.052 (0.047)	40	Exposure assessment surveys in 10 granite sheds to measure compliance; full-shift respirable dust samples in workers' breathing zone and area samples; gravimetric analysis of respirable dust; crystalline silica analysis using OSHA ID 142; TWA exposures calculated
Brown & Rushton (2005a) Industrial silica sand, United Kingdom, 1978–2000	Quarries	Unadjusted geometric mean (GSD) 0.09 (3.9)	2429 (personal) 583 (static)	Samples collected by companies as part of routine monitoring programme; gravimetric analysis; silica content measured by Fourier transform infrared spectrophotometry until 1997 and by X-ray diffraction thereafter; personal and static measurements combined into one data set
Gottesfeld *et al.* (2008) Stone–crushing mills, India, 2003 (initial phase), 2006 and 2007 (post-implementation of engineering controls)		Arithmetic mean (SD)		Bulk and personal air samples collected; silica analysis using NIOSH method 7500; NIOSH method 0500 for respirable particulates used in 2003
	Prior to water-spray controls (2003)	Cristobalite, 0.09 (0.08)	[5]	
		Quartz, 0.25 (0.12)	[5]	
	After water-spray controls			
	Monsoon season (winter 2007)	Cristobalite, 0.02 (0.01)	[18]	
		Quartz, 0.01 (0.01)	[18]	
	Dry season (summer 2006)	Cristobalite, 0.03 (0.03)	[27]	
		Quartz, 0.06 (0.12(	[27]	
Yingratanasuk *et al.* (2002) Stone carvers, Thailand, 1999–2000		Arithmetic mean	148 (total number of samples)	Cross-sectional study design; full-shift (8-hour) personal dust samples; respirable dust analysed gravimetrically; silica analysis by infrared spectrophotometry
	Carvers (Site 1)	0.22		
	Pestle makers (Site 1)	0.05		
	Mortar makers (Site 2)	0.05		
	Mortar makers (Site 3)	0.88		

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m³)	Number of samples	Comments
Rando *et al.* (2001) Industrial sand industry, North America, 1974–98	Sand-processing plants	Geometic mean 0.042 (overall)	14249	Exposure estimates created for a longitudinal and case-referent analysis of a cohort of industrial sand workers; gravimetric analysis of total dust; silica analysis by X-ray diffraction spectroscopy
Yassin *et al.* (2005) Stonework masonry, USA, 1988–2003	All occupations	Geometric mean (GSD) 0.065 (0.732)	274	Analysis of personal silica measurements (n = 7209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
Foundries				
Andersson *et al.* (2009) Iron foundry, Sweden, April 2005–May 2006		Geometric mean (GSD)		Respirable dust, quartz, cristobalite, trydimite samples collected on 2 consecutive workdays for shift and daytime workers; gravimetric analysis conducted using modified NIOSH method; respirable quartz and cristobalite analysed using modified NIOSH method 7500
	Caster	0.020 (1.8)	22	
	Core Maker	0.016 (2.3)	55	
	Fettler	0.041 (2.9)	115	
	Furnace and ladle repair	0.052 (3.7)	33	
	Maintenance	0.021 (2.6)	26	
	Melter	0.022 (2.0)	49	
	Moulder	0.029 (2.6)	64	
	Sand mixer	0.020 (2.3)	14	
	Shake out	0.060 (1.7)	16	
	Transportation	0.017 (2.6)	13	
	Other	0.020 (2.0)	28	
	All occupations	0.028 (2.8)	435	

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m³)	Number of samples	Comments
Yassin *et al.* (2005) Grey–iron foundry, USA 1988–2003		Geometric mean (GSD)		Analysis of personal silica measurements (n = 7 209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
	Spruer	0.154 (0.100)	22	
	Hunter operator	0.093 (1.144)	10	
	Charger	0.091 (0.999)	8	
	Core maker	0.078 (1.033)	89	
	Grinder	0.075 (0.821)	371	
	Molder	0.073 (0.910)	308	
	Abrasive blast operator	0.070 (0.821)	56	
	Sorter	0.067 (0.827)	23	
	Reline cupola	0.067 (0.725)	29	
	Furnace operator	0.066 (0.766)	47	
	Core setter	0.066 (0.671)	23	
	Craneman	0.066 (0.815)	16	
	Cleaning department	0.060 (0.879)	36	
	Inspector	0.057 (1.298)	21	
	Ladle repair	0.055 (0.829)	30	
Other metallurgical operations				
Føreland *et al.* (2008) Silicon carbide industry, Norway, November 2002–December 2003		Geometric mean	720 (total)	Exposure survey conducted in 3 silicon carbide plants; measurements collected to improve previously developed job–exposure matrix; sampling duration close to full shift (6–8 hours); 2 sampling periods of 2 work weeks; gravimetric analysis of respirable dust; silica analysis using modified NIOSH method 7500
	Cleaning operators (Plant A)	0.020 (quartz)		
	Mix operators (Plants A and C), charger/ mix and charger operators (Plant C)	0.008–0.013 (quartz)		
	All other jobs (Plants A, B and C)	< 0.005 (quartz)		
	Charger/mix operators (Plant C)	0.038 (cristobalite)		
Construction				
Tjoe-Nij *et al.* (2003) Construction, the Netherlands		Geometric mean (GSD)		Cross-sectional study design; repeated dust measurements (n = 67) on 34 construction workers; full-shift (6–8 hours) personal respirable dust sampling; gravimetric analysis of respirable dust; silica analysis by infrared spectroscopy (NIOSH method 7602); 8-h TWA concentrations calculated
	Concrete drillers and grinders	0.42 (5.0)	14	
	Tuck pointers	0.35 (2.8)	10	
	Demolition workers	0.14 (2.7)	21	

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m^3)	Number of samples	Comments
Akbar-Khanzadeh & Brillhart (2002) Construction, USA	Concrete–finishing (grinding)	Arithmetic mean (SD) 1.16 (1.36)	49	Task-specific silica exposure assessment conducted as part of an OSHA Consultation Service in Ohio; gravimetric analysis of respirable samples using NIOSH method 0600; silica analysis using in-house method based on NIOSH method 7500 and OSHA ID 142
Verma *et al.* (2003)	Labourers	Range (min–max) 0.10–0.15	20	Task-based exposure assessment conducted as part of an epidemiological study of Ontario construction workers; personal dust sampling and direct–reading particulate monitoring; gravimetric analysis of respirable dust using modified NIOSH method 0600; respirable silica analysis using modified NIOSH method 7500
	Operating engineers	0.04–0.06	3	
	Carpenters, iron workers, masons, painters, terrazzo workers	below detectable limits	17	
Woskie *et al.* (2002) Heavy and highway construction, USA	Labourers	Geometric mean (GSD) 0.026 (5.9)	146	Personal samples collected using the Construction Occupational Health Program sampling strategy; particulate samples analysed gravimetrically; quartz analysed by Fourier transform infrared spectrophotometry; duration of sampling—6 hours of an 8-hour working day
	Miscellaneous trade	0.013 (2.8)	26	
	Operating engineers	0.007 (2.8)	88	
Flanagan *et al.* (2003) Construction, USA, August 2000–January 2001	Clean-up, demolition with hand-held tools, concrete cutting, concrete mixing, tuck-point grinding, surface grinding, sacking and patching concrete, and concrete-floor sanding	Geometric mean (GSD) 0.11 (5.21)	113	Respirable samples analysed gravimetrically using NIOSH method 0600; silica analysed by Fourier transform infrared spectrophotometry using NIOSH method 7602
Lumens & Spee (2001) Construction, the Netherlands	Recess miller	Geometric mean (GSD) 0.7 (3.3)	53	Personal air samples collected during field study at 30 construction sites; duration of sampling 3 to 4 hours; gravimetric analysis of respirable dust samples; silica analysis using NIOSH method 7500
	Demolition worker	1.1 (4.0)	82	
	Inner wall constructor	0.04 (2.6)	36	
	Overall	0.5 (5.6)	171	

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m³)	Number of samples	Comments
Flanagan *et al.* (2006) Construction, USA, 1992–2002	Abrasive blasters, surface and tuck point grinders, jackhammers, rock drills	Geometric mean (GSD) 0.13 (5.9)	1374	Personal silica measurements collected as part of a silica-monitoring compilation project; data provided by 3 federal or state regulatory agencies (n = 827 samples), 6 university or research agencies (n = 491), and 4 private consultants or contractors (n = 134)
Akbar-Khanzadeh *et al.* (2007) Construction, USA	Uncontrolled conventional grinding Wet grinding Local exhaust ventilation grinding	Arithmetic mean 61.7 0.896 0.155	5 sessions 7 sessions 6 sessions	Personal samples collected during grinding operations in a controlled field laboratory to evaluate the effectiveness of wet grinding and local exhaust ventilation; samples collected and analysed using NIOSH methods 0600 and 7500
Bakke *et al.* (2002) Construction, Norway, 1996–99	Tunnel workers	Geometric mean (GSD) α-Quartz, 0.035 (5.0)	299	Personal samples collected as part of exposure survey; sampling duration: 5 to 8 h; respirable dust analysed gravimetrically; silica analysed by NIOSH method 7500
Linch (2002) Construction, USA, 1992–98	Abrasive blasting of concrete structures Drilling concrete highway pavement Concrete-wall grinding Concrete sawing Milling of asphalt	TWA (8-hour) 2.8 3.3 0.26 10.0 0.36		Personal samples collected as part of NIOSH effort to characterize respirable silica exposure in construction industry; respirable dust collected and analysed according to NIOSH method 0600; silica analysed by NIOSH method 7500
Meijer *et al.* (2001) Construction, USA, 1992–93	Concrete workers	Arithmetic mean 0.06	96	Personal samples of respirable dust and silica; gravimetric analysis of respirable dust; silica analysed by infrared spectrophotometry
Miscellaneous operations				
Hicks & Yager (2006) Coal-fired power plants, USA	Normal production activities	Arithmetic mean 0.048	108	Personal breathing zone samples collected during normal full shifts and analysed by NIOSH method 7500

Table 1.3 (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m³)	Number of samples	Comments
Shih *et al.* (2008) Furnace relining, Taiwan, China		Arithmetic mean		Exposures measured in a municipal waste incinerator during annual furnace relining; respirable dust collected and analysed by NIOSH method 0600; silica analysed by NIOSH method 7500
	Sandblasting	0.578	7	
	Bottom-ash cleaning	0.386	8	
	Wall demolishing	0.116	8	
	Relining	0.041	10	
	Grid repairing	0.042	14	
	Scaffold establishing	0.040	8	
	Others	0.082	8	
Zhuang *et al.* (2001) Pottery factories and metal mines, China, 1988–89		Arithmetic mean		Special exposure survey conducted to compare results obtained from traditional Chinese samplers with nylon cyclones; gravimetric analysis of cyclone samples; silica analysis using X-ray diffraction
	Pottery factories	0.116	54	
	Iron/copper mines	0.017	23	
	Tin mines	0.097	10	
	Tungsten mines	0.101	56	
Yassin *et al.* (2005) Several industries, USA, 1988–2003		Geometric mean (GSD)		Analysis of personal silica measurements (n = 7 209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
	Soap and other detergents	0.102 (0.757)	6	
	Testing laboratories services	0.099 (0.896)	53	
	Cut stone and stone products	0.091 (0.956)	405	
	General contractors	0.091 (0.900)	28	
	Coating engraving	0.075 (0.839)	75	
	Grey–iron foundries	0.073 (0.877)	1 760	
	Concrete work	0.073 (0.705)	94	
	Manufacturing explosives	0.070 (0.841)	9	
	Bridge-tunnel construction	0.070 (0.827)	91	
	Stonework masonry	0.065 (0.732)	274	
	Overall	0.073 (0.919)	7209	

GM, geometric mean; GSD, geometric standard deviation; IMIS, Integrated Management Information System; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OSHA; SD, standard deviation

and 40 area samples were collected and analysed by X-ray diffraction. Personal samples were collected after the installation of local exhaust ventilation, and area samples were collected inside the industrial units before (n = 20) and after (n = 20) the installation of local exhaust ventilation. Personal samples were collected from process workers (n = 12), hopper workers (n = 8), drivers (n = 11), and office employees (n = 9). Personal concentrations of respirable dust were as follows: process workers, 0.21 mg/m^3; hopper workers, 0.45 mg/m^3; and, drivers, 0.20 mg/m^3. Personal concentrations of respirable quartz were as follows: process workers, 0.19 mg/m^3; hopper workers, 0.40 mg/m^3; and, drivers, 0.17 mg/m^3. Based on the area samples, the average levels of total dust and respirable dust were 9.46 mg/m^3 and 1.24 mg/m^3, respectively. The amount of free silica in the stone was 85–97%.

Golbabaei *et al.* (2004) measured TWA concentrations of total dust, respirable dust, and crystalline silica (α-quartz) in a marble stone quarry located in the north-eastern region of the Islamic Republic of Iran. Full-shift (2 × 4-hour samples) personal breathing zone samples were collected and analysed using gravimetric and X-ray diffraction methods. The highest levels of total and respirable dust exposure were observed for workers in the hammer drill process area (107.9 mg/m^3 and 11.2 mg/m^3, respectively), and the cutting machine workers had the lowest levels of exposure (9.3 mg/m^3 and 1.8 mg/m^3, respectively). The highest concentrations of α-quartz in total and respirable dust were measured in hammer drill process workers (0.670 mg/m^3 and 0.057 mg/m^3, respectively).

In a NIOSH-conducted cohort mortality study of workers from 18 silica sand plants, Sanderson *et al.* (2000) estimated historical quartz exposures using personal respirable quartz measurements (collected during 1974–96) and impinger dust samples (collected in 1946). During 1974–96, a total of 4269 respirable dust samples were collected from workers performing 143 jobs at these 18 plants. Respirable quartz concentrations ranged from less than 1 to 11700 μg/m^3, with a geometric mean concentration of 25.9 μg/m^3. Over one-third of the samples exceeded the Mine Safety and Health Administration permissible exposure limit value for quartz (PEL, 10 mg/m^3/(%quartz + 2)), and half of the samples exceeded the NIOSH recommended exposure limit [at the time] (REL, 0.050 mg/m^3). Quartz concentrations varied significantly by plant, job, and year and decreased over time, with concentrations measured in the 1970s being significantly greater than those measured later.

(d) Foundries

Lee (2009) reported on exposures to benzene and crystalline silica during the inspection of a foundry processing grey and ductile iron. The facility consisted of two buildings: the main foundry where moulding, core-making, metal pouring, and shakeout took place; and, the finishing part of the site where grinding and painting was done. Personal sampling for crystalline silica was conducted in the grinding area, in casting shakeout, and in both the mould- and core-making operations. Eight-hour TWA concentrations of crystalline silica were in the range of 2.11–4.38 mg/m^3 in the grinding area (n = 4), 1.18–2.14 mg/m^3 in the shakeout area (n = 2), and 1.15–1.63 mg/m^3 in the core-maker area (n = 2). The 8-hour TWA concentration in the mould area was 0.988 mg/m^3.

(e) Construction

In a study of cement masons at six commercial building sites in Seattle, WA, USA, Croteau *et al.* (2004) measured personal exposures to respirable dust and crystalline silica during concrete-grinding activities to assess the effectiveness of a commercially available local exhaust ventilation (LEV) system. Levels were measured with and without LEV, one sample directly after the other. A total of 28 paired

samples were collected. The results showed that the application of LEV resulted in a mean exposure reduction of 92%, with the overall geometric mean respirable dust exposure declining from 4.5 to 0.14 mg/m^3. However, approximately one quarter of the samples collected while LEV was being used were greater than the OSHA 8–hour TWA PEL (22% of samples), and the American Conference of Governmental Industrial Hygiene (ACGIH) threshold limit value (26%) for respirable crystalline silica.

Rappaport *et al.* (2003) investigated exposures to respirable dust and crystalline silica among 80 workers in four trades (bricklayers, painters (when abrasive blasting), operating engineers, and labourers) at 36 construction sites in the Eastern and Midwestern USA. A total of 151 personal respirable air samples were collected and analysed using gravimetric and X-ray diffraction methods. Painters had the highest median exposures for respirable dust and silica (13.5 and 1.28 mg/m^3, respectively), followed by labourers (2.46 and 0.350 mg/m^3), bricklayers (2.13 and 3.20 mg/m^3), and operating engineers (0.720 and 0.075 mg/m^3). The following engineering controls and workplace characteristics were found to significantly affect silica exposures: wet dust suppression reduced labourers' exposures by approximately 3-fold; the use of ventilated cabs reduced operating engineers' exposures by approximately 6-fold; and, working indoors resulted in a 4-fold increase in labourers' exposures.

(f) Agriculture

Archer *et al.* (2002) assessed the exposure to respirable silica of 27 farm workers at seven farms in eastern North Carolina, USA. Four-hour personal breathing zone samples (n = 37) were collected during various agricultural activities and analysed for respirable dust, respirable silica, and percentage silica using gravimetric and X-ray diffraction methods. The overall mean respirable dust, respirable silica, and percentage silica values were 1.31 mg/m^3 (n = 37), 0.66 mg/m^3 (n = 34), and 34.4% (n = 34), respectively. The highest respirable dust and respirable silica concentrations were measured during sweet potato transplanting (mean, 7.6 and 3.9 mg/m^3, respectively; n = 5), and during riding on or driving an uncabbed tractor (mean, 3.1 and 1.6 mg/m^3, respectively; n = 13).

Nieuwenhuijsen *et al.* (1999) measured personal exposure to dust, endotoxin, and crystalline silica during various agricultural operations at ten farms in California, USA, between April 1995 and June 1996. A total of 142 personal inhalable samples and 144 personal respirable samples were collected. The highest levels of inhalable dust exposure were measured during machine-harvesting of tree crops and vegetables (GM, 45.1 mg/m^3 and 7.9 mg/m^3, respectively), and during the cleaning of poultry houses (GM, 6.7 mg/m^3). Respirable dust levels were generally low, except for machine-harvesting of tree crops and vegetables (GM, 2.8 mg/m^3 and 0.9 mg/m^3, respectively). The percentage of crystalline silica was higher in the respirable dust samples (overall, 18.6%; range, 4.8–23.0%) than in the inhalable dust samples (overall, 7.4%; range, not detectable to 13.0%).

(g) Miscellaneous operations

Harrison *et al.* (2005) analysed respirable silica dust samples (n = 47) from several Chinese workplaces (three tungsten mines, three tin mines, and nine pottery mines) to determine the effect of surface occlusion by alumino-silicate on silica particles in respirable dust. The average sample percentages of respirable-sized silica particles indicating alumino-silicate occlusion of their surface were: 45% for potteries, 18% for tin mines, and 13% for tungsten mines.

To provide a more precise estimate of the quantitative relationship between crystalline silica and lung cancer, 't Mannetje *et al.* (2002) conducted a pooled analysis of existing quantitative exposure data for ten cohorts exposed to silica

(US diatomaceous earth workers; Finnish and US granite workers; US industrial sand workers; Chinese pottery workers, and tin and tungsten miners; and South African, Australian, and US gold miners). Occupation- and time-specific exposure estimates were either adopted/adapted or developed for each cohort, and converted to milligrams per cubic metre (mg/m^3) respirable crystalline silica. The median of the average cumulative exposure to respirable crystalline silica ranged from 0.04 mg/m^3 for US industrial sand workers to 0.59 mg/m^3 for Finnish granite workers. The cohort-specific median of cumulative exposure ranged from 0.13 mg/m^3–years for US industrial sand workers to 11.37 mg/m^3–years for Australian gold miners.

In a cross-sectional survey, Hai *et al.* (2001) determined the levels of respirable nuisance and silica dusts to which refractory brickworkers were exposed at a company in Ha Noi, Viet Nam. Respirable dust levels were in the range of 2.2–14.4 mg/m^3 at nine sample sites. The estimated free silica content of dust was 3.5% for unfired materials at the powder collectors (n = 8 samples), and 11.4% in the brick-cleaning area following firing (n = 1 sample).

Burgess (1998) investigated processes associated with occupational exposure to respirable crystalline silica in the British pottery industry during 1930–1995, and developed a quantitative job–exposure matrix. Exposure estimates were derived from 1390 air samples, the published literature, and unpublished reports of dust control innovations and process changes. In the matrix, daily 8-hour TWA airborne concentrations of respirable crystalline silica ranged from 0.002 mg/m^3 for pottery-support activities performed in the 1990s to 0.8 mg/m^3 for firing activities in the 1930s. Although exposure estimates within decades varied, median concentrations for all process categories displayed an overall trend towards progressive reduction in exposure during the 65 year span.

2. Cancer in Humans

2.1 Cancer of the lung

In the previous *IARC Monograph* (IARC, 1997) not all studies reviewed demonstrated an excess of cancer of the lung and, given the wide range of populations and exposure circumstances studied, some non-uniformity of results had been expected. However, overall, the epidemiological findings at the time supported an association between cancer of the lung and inhaled crystalline silica (α-quartz and cristobalite) resulting from occupational exposure.

The current evaluation has a primary focus on studies that employed quantitative data on occupational exposures to crystalline silica dust (α-quartz and cristobalite). The establishment of exposure–response relationships not only provides critical evidence of causation, but the availability of quantitative exposures on crystalline silica and other exposures of relevance facilitates the accurate assessment of exposure–response relationships in the presence of potential confounders. In addition to the focus on quantitative exposure–response relationships, a summary of findings from eight published meta-analyses of lung cancer was also elaborated. Of these, the seven meta-analyses involving absolute risk summarize the information from the many studies that did not consider quantitative exposure–response relationships, while the eighth is a meta-analysis of exposure-response.

Findings from cohort studies are given in Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.1.pdf, and those for the case–control studies are provided in Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.2.pdf. Given that there was concern by the previous IARC Working Group that different exposure settings (including the nature of the industry and the crystalline silica polymorph) may give rise to different (or

no) cancer risks, this evaluation is divided into sections based on the industrial setting where exposure to silica occurs. As with other evaluations, data from community-based studies are not included, although studies of persons with silicosis are.

2.1.1 Diatomaceous earth

Work in the diatomaceous earth industry is associated mainly with exposure to cristobalite rather than quartz, and, in the USA, is generally free of other potential confounding exposures apart from exposure to asbestos in a minority of locations. The first study of US diatomaceous earth workers revealed significant positive trends in lung cancer risk with both cumulative exposure to crystalline silica (semiquantitative) and duration of employment (Checkoway *et al.*, 1993). Owing to concerns with confounding from asbestos, estimates of asbestos exposure were developed (Checkoway *et al.*, 1996). Those with uncertain asbestos exposures were omitted from the analysis leading to the loss of seven lung cancer deaths. Among those with no asbestos exposure, the lung cancer standardized mortality ratios (SMR) for the two higher crystalline silica exposure groups were twice the magnitude of those for the two lowest exposure groups, although they were not significantly elevated. Rate ratios, with and without adjustment for asbestos exposure were very similar (within 2%), indicating that confounding due to asbestos was not an issue. Checkoway *et al.* (1997) provided findings from one of the two plants previously investigated but including 7 more years of follow-up as well as newly developed quantitative respirable crystalline silica exposures (Table 2.1 online). The lung cancer relative risks (RR) for the highest unlagged or 15-year exposure category were both significantly elevated. Trends for both unlagged and lagged exposure–response were of borderline significance. Rice *et al.* (2001) used the same cohort to examine risk, assessing the relationship between lung cancer mortality and respirable crystalline silica exposure using a variety of models. All except one model demonstrated statistical significance, and the trends of the predicted rate ratios with cumulative crystalline silica exposure were generally similar across models.

A small cohort study among Icelandic diatomaceous earth workers (Rafnsson & Gunnarsdóttir, 1997) provided findings that supported an effect of crystalline silica on lung cancer risk (SIR, 2.34; 95%CI: 0.48–6.85 for those who had worked 5 or more years). Smoking habits among the workers were reported to be similar to the general population.

2.1.2 Ore mining

Steenland & Brown (1995) updated a cohort of US gold miners previously studied (McDonald *et al.*, 1978; Table 2.1 online). Using quantitative estimates of cumulative exposure based on particle counts, no obvious evidence of exposure–response with lung cancer mortality was observed, nor were any of the exposure category SMRs elevated. In contrast, tuberculosis and silicosis mortality was elevated and exhibited an exposure–response relationship with crystalline silica exposure.

Gold miners were investigated in a South African cohort study (Hnizdo & Sluis-Cremer, 1991) and in case–control studies nested within that cohort study and within another South African gold miner cohort (Reid & Sluis-Cremer, 1996; Tables 2.1 and 2.2 online). In the Hnizdo & Sluis-Cremer, (1991) cohort study, lung cancer mortality was related to cumulative dust exposure when modelled as a continuous variable (respirable-surface-area–years) adjusting for smoking, as well demonstrating a monotonic increase with categories of cumulative exposures. There was also some indication of exposure–response in both case–control studies: RR, 1.12; 95%CI: 0.97–1.3 for Reid & Sluis-Cremer (1996),

and lung cancer mortality was elevated in the highest exposure group adjusting for smoking in the Hnizdo *et al.* (1997) study. [In this study, exposure to uranium did not confound the results.] [The Working Group noted the potential for confounding from radon, and also noted that the South African cohorts might overlap.]

McLaughlin *et al.* (1992) undertook a nested case–control study of lung cancer among the members of a prior cohort study by Chen *et al.* (1992) (Table 2.2 online). The study included workers from iron, copper, tungsten, and tin mines, and used quantitative estimates of crystalline silica dust and certain confounder exposures. Only tin miners showed a clear and substantial exposure–response relationship with the quantitative measures of crystalline silica cumulative exposure. The tin miners underwent further follow-up in a cohort study (Chen *et al.*, 2006) and a nested case–control study (Chen & Chen, 2002). Although the cohort study findings provided some overall indication of elevated lung cancer exposure–response mortality with cumulative dust exposure (Table 2.1 online), the findings were much less clear when presented by mine and silicosis status. In the nested case–control study (Table 2.2 online), there was evidence of exposure–response with cumulative total dust exposures. There was also evidence of a relationship between lung cancer mortality and cumulative arsenic exposure, but the high correlation between arsenic and crystalline silica levels prevented mutual adjustment, and left the etiological factor unclear. The same conclusions, more generally expressed, were reported in a simple ever/never exposed approach by Cocco *et al.* (2001), and were confirmed by Chen *et al.* (2007) adjusting for smoking and other confounding factors. Here, no relationship of lung cancer mortality with cumulative crystalline silica exposure was noted for the tungsten mines, nor was any evidence for the iron and copper mines adjusting for radon. [The Working Group noted that crystalline silica exposures were very low in the iron and copper mines.] For the tin mines, no adjustment for arsenic could be made because of its collinearity with crystalline silica exposure, but in the overall group, adjusting for smoking, arsenic, polyaromatic hydrocarbons (PAHs), and radon, no exposure–response for cumulative crystalline silica exposure emerged either by quintile or through the use of a continuous predictor. This was especially true when the iron/copper mines were removed for reason of having poorer data, when the trend tended towards lower risk with increasing crystalline silica exposure.

Carta *et al.* (2001) examined 724 compensated silicotics with radiographic indication of 1/0 or greater small opacities on the International Labor Organization scale who had worked at Sardinian lead and zinc mines, brown coal mines, and granite quarries. Using quantitative estimates of cumulative exposure to respirable crystalline silica dust and radon, the exposure–response was studied in a cohort study and a nested case–control study of 34 lung cancer cases (Tables 2.1 and 2.2 online). Little evidence of a trend with crystalline silica exposure was observed in either study component (after controlling for smoking, airflow obstruction, radon, and severity of silicosis in the case–control study). A clear relationship emerged with exposure to radon in the case–control study. [The Working Group noted that this study was small.]

2.1.3 Ceramics

A case–control study of Chinese pottery workers showed evidence of elevated risk for lung cancer with exposure to crystalline silica dust, although no obvious exposure–response was seen in the three higher exposure categories (McLaughlin *et al.*, 1992; Table 2.2 online). This study was nested within the cohort analysis by Chen *et al.* (1992). Although reported exposure to asbestos was to be minimal, the workers were exposed to PAHs, and in a separate analysis

there were non-significant elevations in lung cancer risk with increasing cumulative exposure to PAHs. This was confirmed in the follow-up analysis by Chen *et al.* (2007) that found that the pottery workers had the highest PAH levels over all industrial groups. Adjustment for PAHs in the analysis led to the crystalline silica exposure relative risk of 1.1 (95%CI: 1.02–1.12) dropping to 1.0 (95%CI: 0.96–1.09). [The Working Group noted that in the prior analysis of the Chinese ceramics data by McLaughlin *et al.* (1992), adjusting for PAHs slightly raised rather than reduced the crystalline silica exposure relative risks. The correlation between the crystalline silica and PAH exposures was reported as 0.56.]

Another case–control study of pottery workers with quantitative crystalline silica dust exposures was from the United Kingdom (Cherry *et al.*, 1998). This analysis, which was restricted to ever smokers but adjusted for smoking amount and ex-smoking, showed a significantly elevated risk of lung cancer mortality with increasing average intensity of exposure, but not with cumulative exposure. No confounders, apart from smoking, were noted in this report.

Ulm *et al.* (1999) looked at workers in the German ceramics industry, as well as the stone and quarrying industry. The study was based solely on those without silicosis, as assessed using radiographic appearances. No relationship of lung cancer mortality risk with cumulative exposure, average intensity, nor peak exposure was seen in the ceramic worker subset nor overall. [The Working Group noted that the omission of those with silicosis may have restricted the range of crystalline silica exposure in the analysis leading to a loss of power to detect any relationship between crystalline silica exposure and lung cancer mortality. Moreover, the modelling included duration of exposure along with cumulative exposure, perhaps reducing the ability to detect an effect of crystalline silica exposure.]

2.1.4 Quarries

In an extension of the Vermont granite workers study by Costello & Graham (1988), Attfield & Costello (2004) both lengthened the follow-up from 1982 to 1994, and developed and analysed quantitative crystalline silica dust exposures (Table 2.1 online). The exposures were noteworthy for being developed from environmental surveys undertaken throughout the period of the study. However, information on smoking and silicosis status was lacking, although confounding from other workplace exposures was likely to have been minimal or non-existent. The results showed a clear trend of increasing risk of lung cancer mortality with increasing cumulative respirable crystalline silica exposure up until the penultimate exposure group. [The Working Group noted that the findings were heavily dependent on the final exposure group; when it was included, the models were no longer statistically significant.] Graham *et al.* (2004) undertook a parallel analysis of the same data as Attfield & Costello (2004), but did not use quantitative exposures, and adopted essentially the same analytical approach as in their 1998 study. They concluded that there was no evidence that crystalline silica dust exposure was a risk factor for lung cancer, their main argument being that lung cancer risks were similar by duration and tenure between workers hired pre-1940 and post-1940 – periods before and following the imposition of dust controls when the crystalline silica dust levels were very different.

As noted above, Ulm *et al.* (1999) looked at workers in the German stone and quarrying industry (includes some sand and gravel workers), as well as the ceramics industry (Table 2.2 online). The study was based solely on those without silicosis, as assessed using radiographic appearances. Neither cumulative exposure, average intensity, nor peak exposure showed a relationship with lung cancer risk in the stone and quarry worker subset, nor overall. [The Working Group noted

that the omission of those with silicosis may have restricted the range of crystalline silica exposure in the analysis leading to a loss of power to detect any relationship between crystalline silica exposure and lung cancer mortality. Moreover, the modelling included duration of exposure along with cumulative exposure, perhaps reducing the ability to detect an effect of crystalline silica exposure.] Another study of German stone and quarry workers found an excess of lung cancer (SMR, 2.40), but no relationship between lung cancer mortality and crystalline silica exposure. [The Working Group noted that the cohort study included only 440 individuals with 16 lung cancer cases. It was also restricted to those with silicosis, which was likely to lead to a lack of low exposures, a consequent limited exposure range, and low study power.]

Among studies that did not use quantitative estimates of crystalline silica exposure, that by Koskela *et al.* (1994) is of interest because it reported that the workers had little exposure to possible confounding exposures. The risk of lung cancer was significantly elevated among those with longer duration of exposure and longer latency ($P < 0.05$). Guénel *et al.* (1989) also found an excess of lung cancer among stone workers after adjustment for smoking, but this was not the case in a study of slate workers by Mehnert *et al.* (1990).

2.1.5 Sand and gravel

Confounding from other workplace exposures is minimal in sand and gravel operations. There are three main studies of sand and gravel workers, two in North America and one in the United Kingdom. The North American studies appear to arise from the same population of workers although there is no published information on their overlap, if any. Using the basic information from the McDonald *et al.* (2001) cohort study of nine North American sand and gravel workers, Hughes *et al.* (2001) reported significant exposure–response of lung cancer with quantitative estimates of cumulative respirable crystalline silica exposures and other related indices. McDonald *et al.* (2005) examined a slightly smaller subset of the cohort described by McDonald *et al.* (2001) based on an extended update at eight of the nine plants, and also undertook a nested case–control study. Risk of lung cancer increased monotonically with unlagged cumulative exposure ($P = 0.011$), but 15-year lagged cumulative exposures provided a slightly better fit ($P = 0.006$) (Table 2.2 online). These findings were basically similar to those obtained by Hughes *et al.* (2001) using the larger cohort and shorter follow-up time. McDonald *et al.* (2005) reported that average exposure intensity, but not years employed, showed a relationship with lung cancer risk ($P = 0.015$).

Steenland & Sanderson (2001) studied workers in 18 sand and gravel companies in the same trade organization as the nine included in the McDonald *et al.* (2001) study (Table 2.1 online). They, too, employed quantitative estimates of exposure derived from company records, and found indications of a relationship with lung cancer mortality, most strongly in the subset that had worked 6 or more months in the industry ($P < 0.06$). Further analysis using a nested case–control approach found marginal evidence of exposure–response using quartiles of cumulative exposure ($P = 0.04$), but stronger evidence with average intensity ($P = 0.003$). [The Working Group noted that a sensitivity analysis of the effect of smoking in this cohort (Steenland & Greenland, 2004) led to an adjusted overall SMR estimate of 1.43 (95% Monte Carlo limits: 1.15–1.78) compared with the original SMR of 1.60 (95%CI: 1.31–1.93). The analysis did not deal with the exposure–response estimates.]

The mortality experience of crystalline silica sand workers in the United Kingdom was evaluated by Brown & Rushton (2005b). No overall excess of lung cancer was found (although there was a large, and highly significant, variation

in lung cancer SMRs between quarries; range: 0.27–1.61, both extremes $P < 0.01$. Relative risks rose with cumulative respirable crystalline silica dust exposure in the first two quartiles, but fell below 1.0 in the highest quartile, resulting in no trend being detected. [The Working Group noted that Steenland (2005) commented that the low exposures in the Brown & Rushton (2005b) study was likely to have impacted its power to detect a crystalline-silica effect.]

2.1.6 Other industries

Two studies having quantitative exposures to crystalline silica remain, although both industries are known to be associated with exposure to other known or suspected lung carcinogens. The first, by Watkins *et al.* (2002) was a small case–control study focused on asphalt fumes and crystalline silica exposure. Crystalline silica exposures were low compared to most other studies, and there were no significant lung cancer elevations or trends with exposure (Table 2.2 online). The second study was a nested case–control analysis of Chinese iron and steel workers (Xu *et al.*, 1996). A significant trend with cumulative total dust exposure was reported but not for cumulative crystalline silica dust exposure, although the relative risk for the highest crystalline silica-exposed group was elevated. The findings were adjusted for smoking, but not for benzo[*a*]pyrene exposures, for which the relative risks demonstrated a clear and significant trend with cumulative exposure level.

2.1.7 Semiquantitative exposure and expert-opinion studies

The studies that follow used quantitative exposure measurements in deriving crystalline silica exposure estimates for individuals but ultimately converted them to exposure scores or categories in the epidemiological analysis. Hessel *et al.* (1986) undertook a case–control study of lung cancer and cumulative crystalline silica exposure in South African gold miners after coding the dust measurements to four discrete levels (0, 3, 6, 12). No exposure–response was detected. Neither was any evidence of exposure–response detected in the later necropsy study of South African gold miners (Hessel *et al.*, 1990) that used the same approach to code the exposure data. [The Working Group noted that the study methods in the case–control study may have led to overmatching for exposure in the case–control study, and that there may have been some selection bias and exposure misclassification in the second study.]

de Klerk & Musk (1998) undertook a nested case–control analysis of lung cancer within a cohort study of gold miners and showed exposure–response for log of cumulative exposure (exposure-score–years) but not for any other index of exposure. The analysis adjusted for smoking, bronchitis, and nickel exposures, and took account of asbestos exposure. The study by Kauppinen *et al.* (2003) on road pavers found a relative risk for lung cancer of 2.26 in the highest exposure group, but there was no evidence of a linear trend of risk with level of exposure. No adjustment was made for concomitant exposures to PAHs, diesel exhaust, and asbestos, nor smoking. Moulin *et al.* (2000) conducted a nested case–control study to examine lung cancer among workers producing stainless steel and metallic alloys. Their results on 54 cases and 162 controls, adjusted for smoking but not for other confounders, indicated a marginally significant evidence of a trend with increasing crystalline silica exposure as well as with PAH exposure.

Two population-based studies that involved substantial expert opinion in assigning dust levels in developing quantitative crystalline silica exposures Brüske-Hohlfeld *et al.* (2000) and Pukkala *et al.* (2005) showed an increasing risk of lung cancer with increasing crystalline silica exposure after adjustment for smoking, and in the latter study, also for social class and exposure to asbestos.

2.1.8 Pooled analysis, meta-analyses, and other studies

Steenland *et al.* (2001) reported on a case–control analysis nested within a pooled study of data from ten cohorts from a variety of industries and countries (Table 2.2 online). It included information on diatomaceous, granite, industrial sand, and pottery workers, and workers in tungsten, tin, and gold mines. Results from all of the studies had been previously published, although not all had originally employed quantitative estimates of crystalline silica exposure; and for half, the duration of follow-up had been extended. All indices of cumulative crystalline silica exposure (cumulative, unlagged and lagged; log cumulative, unlagged and lagged) showed highly significant trends with lung cancer risk ($P < 0.0001$), and average exposure demonstrated a less significant trend ($P < 0.05$). Of these indices, log cumulative exposure led to homogeneity in findings across the cohorts ($P = 0.08$ and 0.34 for unlagged and 15-year lag respectively). Findings were similar for the mining and non-mining subgroups. No adjustment was made for smoking and other confounders, although it was noted that smoking had previously been shown not to be a major confounder in five of the ten studies. Analyses of subsets of the data omitting cohorts with suspected other confounders (radon in South African gold mines, and arsenic or PAHs in Chinese tin miners and pottery workers) did not change the overall findings. [The Working Group noted that the robustness in the findings to exclusion of cohorts with potential confounders from other occupational exposures indicates that any confounding in the individual studies were unlikely to have had an impact on their findings related to crystalline silica.]

The presence of silicosis in an individual is a biomarker of high exposure to crystalline silica dust. Accordingly, studies of individuals with silicosis have the potential to provide useful information on the lung cancer risk associated with exposure to crystalline silica. Three meta-analyses have focused on the risk of lung cancer among individuals with silicosis (Smith *et al.*, 1995; Tsuda *et al.*, 1997; Lacasse *et al.*, 2005). Erren *et al.* (2009) also provide summary information in an electronic supplement to their article. Four others have looked at crystalline silica exposure (including silicosis status unknown and those without silicosis; Steenland & Stayner, 1997; Kurihara & Wada, 2004; Pelucchi *et al.*, 2006; Erren *et al.*, 2009). The number of studies included ranged from 11 in a meta-analysis focused on individuals without silicosis (Erren *et al.*, 2009) to 43 (Pelucchi *et al.*, 2006) in a study of those with and without silicosis. Reasons for this variation included: the publication date, the time period of interest, whether the study was focused on those with or without silicosis, the originating country of the studies, and analysis-specific criteria. For example, Steenland & Stayner (1997) rejected studies of miners and foundry workers on the assumption that they had the greatest potential for confounding exposures, and Smith *et al.* (1995) rejected certain studies they deemed under or overestimated the risk of lung cancer. Overall, of the total of 112 publications included by one or more of the seven meta-analyses, none were common to all analyses.

The detailed results from the seven meta-analyses are shown in Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.3.pdf. In brief, all analyses except for those devoted to categories without silicosis found an elevated lung cancer risk, whether occurring among those with silicosis or among crystalline-silica-exposed workers, or arising from cohort or case–control studies. [The Working Group noted that studies that restrict their analysis to individuals without silicosis potentially limit their range of crystalline silica exposure, because individuals with the highest exposures tend to be omitted. Limiting the range of exposure results in reduced power to detect associations.] Overall, the rate ratios were

very similar across studies (1.74–2.76 for those with silicosis, and 1.25–1.32 for workers exposed to crystalline silica). Results from case–control studies, where there is greater opportunity to control for smoking, revealed lower rate ratios than from cohort studies in two analyses, greater rate ratios in two, and about the same in the fifth (the sixth analysis did not break the results out separately by study type). Moreover, the supplementary materials of Erren *et al.* (2009) show equal risk for crystalline silica exposure in unadjusted and smoking-adjusted studies. The two available analyses providing results on workers exposed to crystalline silica by type of study reported larger rate ratios from the case–control studies.

A further meta-analysis examined exposure–response (Lacasse *et al.*, 2009) rather than overall risk, and for this reason its findings are reported separately. The analysis included findings from ten studies having quantitative measurements of crystalline silica exposure and adjustment for smoking. An increasing risk of lung cancer was observed with increasing cumulative exposure to crystalline silica above a threshold of 1.84 mg/m^3 per year. Although the overall findings were heterogeneous, they were similar to those from a subset of seven more homogeneous studies.

Many of the meta-analyses noted that a lung cancer risk was apparent either after adjusting for smoking or among non-smokers (Smith *et al.*, 1995; Tsuda *et al.*, 1997; Kurihara & Wada, 2004; Lacasse *et al.*, 2005). Yu & Tse (2007) further explored the issue of smoking on the interpretation of the published cohort and case–control studies of crystalline silica exposure and lung cancer. In this, they examined reported SMRs and standardized incidence ratios (SIR) for lung cancer reported in ten different published studies, and concluded that the risk had been systematically underreported for never smokers. After adjustment, five of the ten SMRs and SIRs showed significant lung cancer excesses among never smokers compared to two when unadjusted, and ranged from 2.60–11.93. The SMRs and SIRs for ever smokers were reduced after adjustment for smoking, but tended to retain their statistical significance.

2.2 Other cancers

2.2.1 Cancer of the stomach

In the 40 reports with information on cancer of the stomach, 18 had relative risks > 1.0 (including three significantly elevated), and 22 with relatives risks ≤ 1.0 (including two significantly reduced).

2.2.2 Digestive, gastro-intestinal, or intestinal cancers

In the 15 reports of digestive, gastro-intestinal, or intestinal cancer, seven had relative risks > 1.0 (including one significantly elevated), and eight with reltaive risks ≤ 1.0 (two significantly reduced).

2.2.3 Cancer of the oesophagus

In the 14 reports of oesophageal cancer, five had relative risks > 1.0 (including three significantly elevated), and nine with relative risks ≤ 1.0.

Wernli *et al.* (2006) reported a hazard ratio of 15.80 (95%CI: 3.5–70.6) among Chinese textile workers exposed for over 10 years to crystalline silica dust. In Chinese refractory brick workers, Pan *et al.* (1999) found not only a significant elevation with being ever exposed to crystalline silica dust (RR, 2.75; 95%CI: 1.44–5.25), but also a clear exposure–response relationship with years of exposure, adjusting for smoking and other personal factors. [The Working Group noted that confounding from exposure to PAHs could not be ruled out in the above two studies.]

Yu *et al.* (2007) reported an overall SMR for cancer of the oesophagus of 2.22 (95%CI: 1.36–3.43), and an SMR of 4.21 (95%CI: 1.81–8.30)

among caisson workers (who were noted to have had higher exposures to crystalline silica dust than non-caisson workers). The relative risk of oesophageal cancer for caisson workers with silicosis was reduced to 2.34 after adjusting for smoking and alcohol drinking. No excess risk of oesophageal cancer was observed among the non-caisson workers with silicosis after adjustment.

2.2.4 Cancer of the kidney

In the eight reports on cancer of the kidney, five had relative risks > 1.0 (including two significantly elevated), and three with relative risks ≤ 1.0. The two with significantly elevated risks provided information on exposure–response relationships with crystalline silica exposure, although neither formally evaluated this. In US sand and gravel workers (McDonald *et al.*, 2005), a non-significant negative trend with increasing crystalline silica exposure was observed. However, in Vermont granite workers (Attfield & Costello, 2004), kidney cancer SMRs increased almost monotonically with increasing exposure (except for the last exposure group), and the SMR of 3.12 in the penultimate exposure group was significantly elevated.

2.2.5 Others

There have been isolated reports of excesses in other cancers but the evidence is, in general, too sparse for evaluation. Elci *et al.* (2002) reported an excess of cancer of the larynx in workers potentially exposed to crystalline silica dust, particularly for supraglottic cancer (OR, 1.8; 95%CI: 1.3–2.3), with a significant exposure–response relationship.

2.3 Synthesis

Findings of relevance to lung cancer and crystalline silica exposure arise from five main industrial settings: ceramics, diatomaceous earth, ore mining, quarries, and sand and gravel. Of these, the industries with the least potential for confounding are sand and gravel operations, quarries, and diatomaceous earth facilities. Among those industry segments, most studies with quantitative exposures report associations between crystalline silica exposure and lung cancer risk. The findings are supported by studies in these industries that lack quantitative exposures. Results from the other industry segments generally added support although some studies had potential confounding from arsenic, radon, or PAHs. In one case among Chinese tin miners, the arsenic and crystalline silica exposures were virtually collinear, and no adjustment could be made for arsenic. In another (Chinese pottery workers), adjustment for PAHs removed a significant crystalline silica exposure effect, and in a third, among iron and copper miners, the crystalline silica effect disappeared after adjustment for radon. In these, the role of crystalline silica exposure must be regarded as unclear. Mixed findings were reported among gold, tungsten, and lead/zinc miners.

The strongest evidence supporting the carcinogenicity of crystalline silica in the lung comes from the pooled and meta-analyses. The pooled analysis demonstrated clear exposure–response, while all of the meta-analyses strongly confirmed an overall effect of crystalline silica dust exposure despite their reliance on different studies in coming to their conclusions.

Cancers other than that of the lung have not been as thoroughly researched. In many cases the findings were reported in passing, in analyses focused on lung cancer, and rarely have the data examined exposure–response with crystalline silica exposure or its surrogates.

3. Cancer in Experimental Animals

No additional relevant cancer bioassays have been conducted since the previous *IARC Monograph* (IARC, 1997) except for a study in hamsters by inhalation (Muhle *et al.*, 1998), and a study in mice by intratracheal instillation (Ishihara *et al.*, 2002). Studies from the previous evaluation considered adequate are summarized below together with the new studies published since.

3.1 Inhalation exposure

See Table 3.1

3.1.1 Mouse

Female BALB/cBYJ mice exposed to crystalline silica by inhalation (Wilson *et al.*, 1986) did not have an increase in lung tumours compared to controls. Pulmonary adenomas were observed in both the silica-exposed (9/60) and the control animals (7/59). [The Working Group noted that the study groups were small (6–16 mice).]

3.1.2 Rat

Male and female F344 rats were exposed to 0 or 52 mg/m^3 of crystalline silica (Min-U-Sil) over a 24-month period. Interim removals of ten males and ten females per group were made after 4, 8, 12, and 16 months of exposure. Half of those removed were necropsied, and half were held until the end of the 24 months. None of the controls developed a lung tumour. In the silica-exposed rats, the first pulmonary tumour appeared at 494 days, and the incidence was 10/53 in females and 1/47 in males (Dagle *et al.*, 1986).

One group of 62 female F344 rats was exposed by nose-only inhalation to 12 mg/m^3 crystalline silica (Min-U-Sil) for 83 weeks. An equal number of controls was sham-exposed to filtered air, and 15 rats were left untreated. The animals were observed for the duration of their lifespan. There were no lung tumours in the sham-exposed group, and 1/15 unexposed rats had an adenoma of the lung. In the quartz-exposed rats, the incidence of lung tumours was 18/60 (Holland *et al.*, 1983, 1986; Johnson *et al.*, 1987).

Groups of 50 male and 50 female viral antibody-free SPF F344 rats were exposed by inhalation to 0 or 1 mg/m^3 silica (DQ12; 87% crystallinity as quartz) for 24 months. The rats were then held for another 6 weeks without exposure. The incidence of lung tumours in the silica-exposed rats was 7/50 males and 12/50 in females. Only 3/100 controls had lung tumours (Muhle *et al.*, 1989, 1991, 1995).

Three groups of 90 female Wistar rats, 6–8 weeks old, were exposed by nose-only inhalation to 6.1 or 30.6 mg/m^3 DQ12 quartz for 29 days. Interim sacrifices were made immediately after the exposure and at 6, 12, and 24 months, with the final sacrifice at 34 months after exposure. The total animals with lung tumours was 0 (controls), 37/82 (low dose), and 43/82 (high dose). Many animals had multiple tumours (Spiethoff *et al.*, 1992).

3.1.3 Hamster

Groups of 50 male and 50 female Syrian golden hamsters were exposed to 0 (control) or 3 mg/m^3 DQ12 quartz (mass median aerodynamic diameter, 1.3 µm) for 18 months. The experiment was terminated 5 months later. In the silica-exposed group, 91% of the animals developed very slight to slight fibrosis in the lung, but no significant increase of lung tumours was observed (Muhle *et al.*, 1998)

Table 3.1 Studies of cancer in experimental animals exposed to crystalline silica (inhalation exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start Particle size, GSD	Incidence of tumours in respiratory tract	Significance	Comments
Mouse, BALB/c BYJ (F) 150, 300 or 570 d Wilson *et al.* (1986)	0, 1.5, 1.8 or 2.0 mg/m^3 8 h/d, 5 d/wk 6–16 animals Diameter < 2.1 µm	Lung (adenomas): 7/59 (control), 9/60 (all exposed)	[NS]	
Rat, F344 (M, F) 24 mo Dagle *et al.* (1986)	0, 52 mg/m^3 6 h/d, 5 d/wk 72/sex MMAD, 1.7–2.5 µm; GSD, 1.9–2.1	Lung (epidermoid carcinomas): M–0/42 (control), 1/47 F–0/47 (control), 10/53	[NS] [$P < 0.002$]	
Rat, F344 (F) Lifespan Holland *et al.* (1983, 1986); Johnson *et al.* (1987)	0, 12 mg/m^3 6 h/d, 5 d/wk for 83 wk 62 animals MMAD, 2.24 µm; GSD, 1.75	Lung (tumours): 0/54 (control), 18/60 (11 adenocarcinomas, 3 squamous cell carcinomas, 6 adenomas)	[$P < 0.001$]	Nose-only inhalation exposure. Age unspecified
Rat, SPF F344 (M, F) 30 mo Muhle *et al.* (1989, 1991, 1995)	0, 1 mg/m^3 6 h/d, 5 d/wk for 24 mo 50/sex MMAD, 1.3 µm; GSD, 1.8	Lung (tumours): 3/100 (control M, F), 7/50 (M), 12/50 (F) M–1 adenoma, 3 adenocarcinomas, 2 benign cystic keratinizing squamous cell tumours, 1 adenosquamous carcinoma, 1 squamous cell carcinoma F–2 adenomas, 8 adenocarcinomas, 2 benign cystic keratinizing squamous cell tumours	Unspecified (M) [$P < 0.05$] (F)	
Rat, Wistar (F) Up to 35 mo Spiethoff *et al.* (1992)	0, 6.1, 30.6 mg/m^3 6 h/d, 5 d/wk for 29 d 90 animals MMAD, 1.8 µm; GSD, 2.0	0/85 (control), 37/82 (low dose), 43/82 (high dose) Multiple tumours/rat: 21 bronchiolo-alveolar adenomas, 43 bronchiolo-alveolar carcinomas, 67 squamous cell carcinomas, 1 anaplastic carcinoma	[$P < 0.0001$] (both doses)	Nose-only inhalation exposure

d, day or days; F, female; GSD, geometric standard deviation; h, hour or hours; M, male; MMAD, mass median aerodynamic diameter; mo, month or months; NS, not significant; wk, week or weeks

3.2 Intranasal administration

3.2.1 Mouse

Two groups of 40 female (C57xBALB/c) F_1 mice received a single intranasal instillation of 4 mg of synthetic *d*- or *l*-quartz. A group of 60 females received an intranasal instillation of saline. Survivors were killed at 18 months after treatment, and the incidence of lymphomas and leukaemias combined was 0/60 (controls), 2/40 (*d*-quartz), and 6/40 (*l*-quartz) (Ebbesen, 1991). [The Working Group noted that the study was not designed to detect silica-induced lung tumours, and also noted the lack of information on quartz retention.]

3.3 Intratracheal administration

See Table 3.2

3.3.1 Mouse

A group of 30 male A/J mice, 11–13 weeks old, received weekly intratracheal instillations of 2.9 mg quartz for 15 weeks. A group of 20 mice received instillations of vehicle [unspecified]. Animals were killed 20 weeks after the instillations. The incidences of lung adenomas were 9/29 in the controls, and 4/20 for the silica-instilled mice, values that were not statistically different (McNeill *et al.*, 1990).

Ishihara *et al.* (2002) administered a single dose (2 mg in saline/mouse) of crystalline silica to a group of four C57BL/6N mice by intratracheal instillation to study subsequent genotoxic effects. A control group of four animals was instilled saline only. Silicotic lesions were observed in the lungs of the exposed mice, but no pulmonary neoplasms were observed after 15 months.

3.3.2 Rat

A group of 40 Sprague Dawley rats [sex unspecified] received weekly instillations of 7 mg quartz (Min-U-Sil) in saline for 10 weeks. Another groups of 40 rats received instillations of saline alone, and 20 rats remained untreated. Animals were observed over their lifespan. The incidence of lung tumours in quartz-treated rats was 6/36, 0/40 in the saline controls, and 0/18 in the untreated rats (Holland *et al.*, 1983).

Groups of 85 male F344 rats received a single intratracheal instillation of 20 mg quartz in deionized water, Min-U-Sil or novaculite, into the left lung. Controls were instilled with vehicle only. Interim sacrifices of ten rats were made at 6, 12, and 18 months with a final sacrifice at 22 months. The incidence of lung tumours in the Min-U-Sil-instilled rats was 30/67, in the novaculite-treated rats 21/72, and in controls 1/75. All of the lung tumours were adenocarcinomas, except for one epidermoid carcinoma in the novaculite-treated rats (Groth *et al.*, 1986).

Groups of male and female F344/NCr rats [initial number unspecified] received one intratracheal instillation of 12 or 20 mg quartz in saline or 20 mg of ferric oxide (non-fibrogenic dust) in saline. Interim sacrifices were made at 11 and 17 months with a final sacrifice at 26 months. There was a group of untreated controls observed at unscheduled deaths after 17 months. No lung tumours were observed in the ferric-oxide-treated rats and only one adenoma was observed in the untreated controls. The high incidences of benign and mainly malignant lung tumours observed in the quartz-treated rats is summarized in Table 3.3 (Saffiotti, 1990, 1992; Saffiotti *et al.*, 1996).

Six groups of 37–50 female Wistar rats, 15 weeks old, received either a single or 15 weekly intratracheal instillation of one of three types of quartz preparations in saline (see Table 3.4). A control group received 15 weekly instillations of saline. To retard the development of silicosis,

Table 3.2 Studies of cancer in experimental animals exposed to silica (intratracheal instillation)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start Particle size	Incidence of tumours	Significance
Mouse, A/J (M) 20 wk McNeill *et al.* (1990)	0, 2.9 mg Weekly for 15 wk 30, 20 (controls) 1–5 µm (size not further specified)	Lung (adenomas): 9/29 (control), 4/20 Tumour multiplicity: 0.31 ± 0.09 (control), 0.20 ± 0.09	[NS] [NS]
Rat, Sprague Dawley (NR) Lifespan Holland *et al.* (1983)	0 (saline), 7 mg Weekly for 10 wk 40 animals 1.71 ± 1.86 µm	Lung (1 adenoma, 5 carcinomas): 0/40 (control), 6/36	[P<0.05] (carcinomas)
Rat, F344 (M) 22 mo Groth *et al.* (1986)	0, 20 mg once only 85 animals < 5 µm	Lung (adenocarcinomas): 1/75 (control), 30/67	[P<0.001]
Rat, F344/NCr (M, F) 11, 17 or 26 mo Saffiotti (1990, 1992); Saffiotti *et al.* (1996)	0, 12, 20 mg quartz Once only Ferric oxide (20 mg) was negative control [Initial number of rats, NR] 0.5–2.0 µm	High incidences of benign and mainly malignant lung tumours in quartz-treated rats reported in Table 3.3 No tumours observed in ferric oxide group One adenoma in untreated controls	NR
Rat, Wistar Lifespan Pott *et al.* (1994)	0 (saline), one single or 15 weekly injections of one of 3 types of quartz Some rats received PVNO to protect against silicosis 37–50/group	Incidences of benign and malignant lung tumours in quartz-treated rats are shown in Table 3.4 No tumours observed in saline-treated rats	NR
Hamster Syrian Golden (NR) Lifespan Holland *et al.* (1983)	0 (saline), 3, 7 mg quartz (Min-U-Sil) Once a wk for 10 wk 48/group; 68 (controls) 1.71 ± 1.86 µm	No lung tumours in any group	
Hamster, Syrian Golden (M) Lifespan Renne *et al.* (1985)	0 (saline), 0.03, 0.33, 3.3, or 6.0 mg quartz (Min-U-Sil) weekly for 15 wk 25–27/group MMAD, 5.1 µm Geometric diameter, 1.0 µm	No lung tumours in any group	
Hamster, Syrian Golden (M) 92 wk Niemeier *et al.* (1986)	0 (saline), 1.1 (Sil-Co-Sil) or 0.7 (Min-U-Sil) mg One group received 3 mg ferric oxide 50/group 5 µm (Min-U-Sil)	No tumours in saline controls or in Sil-Co-Sil groups 1 adenosquamous carcinoma of the bronchi and lung in Min-U-Sil group and 1 benign tumour of the larynx in ferric oxide group	

M, male; MMAD, mass median aerodynamic diameter; mo, month or months; NR, not reported; NS, not significant; PVNO, polyvinylpyridine-*N*-oxide; wk, week or weeks

Table 3.3 Incidence, numbers, and histological types of lung tumours in F344/NCr rats after a single intratracheal instillation of quartz

Treatment		Observation time	Lung tumours	
Material	**Dose**[a]		**Incidence**	**Types**
Males				
Untreated	None	17–26 mo	0/32	
Ferric oxide	20 mg	11–26 mo	0/15	
Quartz (Min-U-Sil 5)	12 mg	Killed at 11 mo	3/18 (17%)	6 adenomas, 25 adenocarcinomas, 1 undifferentiated carcinoma, 2 mixed carcinomas, 3 epidermoid carcinomas
		Killed at 17 mo	6/19 (32%)	
		17–26 mo	12/14 (86%)	
Quartz (HF-etched Min-U-Sil 5)	12 mg	Killed at 11 mo	2/18 (11%)	5 adenomas, 14 adenocarcinomas, 1 mixed carcinoma
		Killed at 17 mo	7/19 (37%)	
		17–26 mo	7/9 (78%)	
Females				
Untreated	None	17–26 mo	1/20 (5%)	1 adenoma
Ferric oxide	20 mg	11–26 mo	0/18	
Quartz (Min-U-Sil 5)	12 mg	Killed at 11 mo	8/19 (42%)	2 adenomas, 46 adenocarcinomas, 3 undifferentiated carcinomas, 5 mixed carcinomas, 3 epidermoid carcinomas
		Killed at 17 mo	10/17 (59%)	
		17–26 mo	8/9 (89%)	
	20 mg	17–26 mo	6/8 (75%)	1 adenoma, 10 adenocarcinomas, 1 mixed carcinoma, 1 epidermoid carcinoma
Quartz (HF-etched Min-U-Sil 5)	12 mg	Killed at 11 mo	7/18 (39%)	1 adenoma, 36 adenocarcinomas, 3 mixed carcinomas, 5 epidermoid carcinomas
		Killed at 17 mo	13/16 (81%)	
		17–26 mo	8/8 (100%)	

[a] Suspended in 0.3 or 0.5 mL saline

HF, hydrogen fluoride; mo, month or months

From Saffiotti (1990, 1992), Saffiotti *et al.* (1996)

Table 3.4 Incidence, numbers, and histological types of lung tumours in female Wistar rats after intratracheal instillation of quartz

Material	Surface area	No. of instillations	No. of rats examined	No. and #% of rats with primary epithelial lung tumours[a]					Other tumours[b]
	(m^2/g)	(del # × mg)		Adenoma	Adenocarcinoma	Benign CKSCT	Squamous cell carcinoma	Total (%)	
Quartz (DQ 12)	9.4	15 × 3	37	0	1z	11	1 + 1y	38	1
Quartz (DQ 12) + PVNO	9.4	15 × 3	38	0	1 + 3z	8 + 1x	4+1x+3y+1z	58	2
Quartz (DQ 12)	9.4	1 × 45	40	0	1	7	1	23	2
Quartz (Min-U-Sil)	–	15 × 3	39	1	4 + 4z	6	1+2y+2z+1y,z	54	3
Quartz (Min-U-Sil) + PVNO	–	15 × 3	35	1	2 + 1x	8	5+1x+1y+1z	57	3
Quartz Sykron (F 600)	3.7	15 × 3	40	0	3	5	3 + 1z	30	1
0.9% Sodium chloride	–	15 × 0.4 mL	39	0	0	0	0	0	5

[a] If an animal was found to bear more than one primary epithelial lung tumour type, this was indicated as follows:[x]adenoma; [y]adenocarcinoma; [z]benign CKSCT.

[b] Other types of tumours in the lung: fibrosarcoma, lymphosarcoma, mesothelioma or lung metastases from tumours at other sites

PVNO, polyvinylpyridine-*N*-oxide; CKSCT, cystic keratinizing squamous cell tumour

From Pott *et al.* (1994)

two of the groups received injections of polyvinylpyridine-*N*-oxide. All groups of quartz-exposed rats had a significant increase in lung tumours, and the rats protected against silicosis developed more pulmonary squamous cell carcinomas than rats that were not protected (Pott *et al.*, 1994).

3.3.3 Hamster

Two groups of 48 Syrian hamsters [sex unspecified] received intratracheal instillations of 3 or 7 mg quartz (Min-U-Sil) in saline once a week for 10 weeks. A group of 68 hamsters received saline alone, and another group of 72 hamsters were untreated. All animals were observed for their lifespan. No lung tumours were observed in any of the groups (Holland *et al.*, 1983).

Groups of 25–27 male Syrian golden hamsters, 11–weeks old, received weekly intratracheal instillation of 0.03, 0.33, 3.3, or 6.0 mg quartz (Min-U-Sil) in saline for 15 weeks. Groups of 27 saline-instilled hamsters and 25 untreated controls were used as controls. Animals were observed for their lifespan. No lung tumours were observed in any group (Renne *et al.*, 1985).

Three groups of 50 male Syrian golden hamsters received weekly instillations of 1.1 mg of quartz as Sil-Co-Sil, or 0.7 mg of quartz as Min-U-Sil, or 3 mg of ferric oxide (non-fibrogenic particle) in saline for 15 weeks. A group of 50 saline-instilled hamsters served as controls. Survivors were killed at 92 weeks after the beginning of the instillations. No respiratory tract tumours were observed in the hamsters exposed to Sil-Co-Sil or in the saline controls. One adenosquamous carcinoma of the bronchi and lung was observed in the Min-U-Sil group, and one benign tumour of the larynx in the ferric-oxide-exposed group (Niemeier *et al.*, 1986).

3.4 Intrapleural and intrathoracic administration

3.4.1 Mouse

One mouse study was reported in the previous *IARC Monograph* (IARC, 1997) in which the route of exposure was via a single intrathoracic injection of tridymite. The study was only reported as an abstract, and therefore is not described here (Bryson *et al.*, 1974).

3.4.2 Rat

Two groups of 48 male and 48 female standard Wistar rats and two groups 48 male and 48 female SPF Wistar rats were given a single intrapleural injection of 20 mg alkaline-washed quartz (size, < 5 µm) in saline, and observed for their lifespan. Control rats received injections of 0.4 mL saline alone. Malignant tumours of the reticuloendothelial system involving the thoracic region were observed in 39/95 quartz-treated SPF rats [$P < 0.001$] (23 histiocytic lymphomas, five Letterer-Siwe/Hand-Schüller-Christian disease-like tumours, one lymphocytic lymphoma, four lymphoblastic lymphosarcomas, and six spindle cell sarcomas), and in 31/94 quartz-treated standard rats [$P < 0.001$] (30 histiocytic lymphomas and one spindle-cell sarcoma). In the SPF control animals, 8/96 rats had tumours (three lymphoblastic lymphosarcomas, five reticulum cell sarcomas), 7/85 standard rat controls had tumours (one lymphoblastic lymphosarcoma, and six reticulum cell sarcomas) (Wagner & Berry, 1969; Wagner, 1970; Wagner & Wagner, 1972). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

In a second study, with the same dosing regimen and type of quartz, 23 rats developed malignant reticuloendothelial system tumours (21 malignant lymphomas of the histiocytic type [MLHT], two thymomas, and one lymphosarcoma/thymoma/spindle cell sarcoma) in 80 male

and 80 female SPF Wistar rats after 120 weeks. In another experiment, 16 male and 16 female SPF Wistar rats dosed similarly with Min-U-Sil quartz were observed until they were moribund. Eight of the 32 rats developed MLHT and three developed thymomas/lymphosarcomas. In a last experiment with the same experimental design, 18 of 32 SPF Wistar rats that had been injected with cristobalite developed malignant lymphomas (13 MLHT and five thymomas/lymphosarcomas). No MLHT and one thymoma/lymphosarcoma tumours were observed in 15 saline-injected control rats. (Wagner, 1976). [The Working Group noted that the distribution of tumours over sexes was unspecified, and that no statistics were provided.]

In one experiment, groups of 16 male and 16 female Wistar rats were given intrapleural injections of 20 mg of four types of quartz (Min-U-Sil, D&D, Snowit, and DQ12). The animals were observed for their lifespan. For all but the group treated with DQ12 quartz, there was a statistically significant increase in MLHT over saline controls (Table 3.5). In a second experiment with the same experimental design, two other strains of rats were injected Min-U-Sil (12 male and 12 female PVG rats and 20 male and 20 female Agus rats). A non-significant increase in MLHT was observed in both strains, and there was no MLHT in the saline controls. In a third experiment with the same experimental design, cristobalite was injected, and 4/32 treated Wistar rats developed MLHT [not significant], but none of the 32 saline controls did. In a final experiment, 16 male and 16 female Wistar rats were injected triolymite (size, < 0.5 μm; 0.35×10^6 particle/μg), and observed for their lifespan. A total of 16/32 Wistar rats developed MLHT, whereas no such tumours were observed in the 32 saline controls (Wagner *et al.*, 1980). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

Two groups of 36 2-month-old male Sprague-Dawley rats, received a single intrapleural injection of 20 mg DQ12 quartz in saline or saline alone, and were observed for their lifespan. Twenty-seven male rats served as untreated controls. Six malignant histiocytic lymphomas and two malignant Schwannomas were observed in the quartz-treated group [not significant], and one chronic lymphoid leukaemia and one fibrosarcoma were observed in the saline group and untreated controls, respectively (Jaurand *et al.*, 1987).

3.5 Intraperitoneal administration

3.5.1 Rat

Two groups of 16 male and 16 female SPF Wistar rats received a single intraperitoneal injection of 20 mg of Min-U-Sil quartz in saline, and were observed for their lifespan. There were 12 saline controls [sex unspecified]. A total of 9/64 quartz-exposed rats developed malignant lymphomas (two MLHT and seven thymoma/lymphosarcomas). None of the saline controls developed MLHT, but one thymoma/lymphosarcoma was noted (Wagner, 1976). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

3.6 Subcutaneous administration

3.6.1 Mouse

Two groups of 40 female (C57xBALB/c) F_1 mice received a single subcutaneous injection of 4 mg of *d*- or *l*-quartz. A group of 60 female mice served as saline controls. At 18 months after injection, there was an incidence of lymphomas/leukemias of 0/60, 1/40 and 12/40 ($P < 0.001$), and of liver adenomas of 0/60, 1/40 and 3/40 for the saline controls, *d*-quartz and *l*-quartz groups, respectively. No injection-site tumours were reported (Ebbesen, 1991).

Table 3.5 Incidences of malignant lymphoma of the histiocytic type (MLHT) in Wistar rats after an intrapleural injection of 20 mg quartz/animal

Sample	No. of particles × 10^6/µg	Size distribution (%)			Mean survival (days)	Incidence of MLHT (%)[a]
		< 1 µm	1–2 µm	2–4.6 µm		
Min-U-Sil (a commercially prepared crystalline quartz probably 93% pure)	0.59	61.4	27.9	9.1	545	11/32 (34%)b
D&D (obtained from Dowson & Dobson, Johannesburg, pure crystalline quartz)	0.30	48.4	33.2	18.4	633	8/32 (25%)b
Snowit (commercially prepared washed crystals)	1.1	81.2	12.9	5.6	653	8/32 (25%)b
DQ12 (standard pure quartz)	5.0	91.4	7.8	0.8	633	5/32 (16%)
Saline controls	–	–	–	–	717	0 [0/32] (0%)

[a] Sex unspecified
[b] [Significantly different from controls by Fisher Exact test, $P < 0.05$]
From Wagner *et al.* (1980)

3.7 Intravenous administration

3.7.1 Mouse

Groups of 25 male and 25 female strain A mice were injected in the tail vein with 1 mg quartz in 0.1 mL of saline, with a control group of 75 male and female untreated animals. Animals were killed 3, 4.5 or 6 months after injection. There was no difference in the incidences and multiplicities of pulmonary adenomas between treated and untreated animals (Shimkin & Leiter, 1940).

3.8 Administration with known carcinogens

3.8.1 Inhalation

(a) Rat

Studies have been completed to determine the effect of co-exposure to silica and Thorotrast, a known carcinogen (See Table 3.6). Two sets of three groups of 90 female Wistar rats, 6–8 weeks old, were exposed by inhalation to 0, 6, or 31 mg/m^3 of DQ12 quartz (mass median diameter, 1.8 µm; GSD, 2.0) for 6 hours/day 5 days/week for 29 days. One week after the inhalation exposure, one group of quartz-exposed rats and one group of sham-exposed rats received an intravenous injection of Thorotrast (2960 Bq ^{228}Th/mL, 0.6 mL). Controls were only sham-exposed. In each of the six groups, interim sacrifices of three or six animals each were made 0, 6, 12 and 24 months after the end of exposure. The experiment was terminated 34 months after the end of exposure. In rats that were exposed to silica by inhalation and then given Thorotrast, there was a small increase in lung tumours compared to the already high incidence of benign and malignant tumours induced by silica alone (Spiethoff *et al.*, 1992).

3.8.2 Intratracheal administration

(a) Rat

Four groups of white rats (group sizes varied from 28 to 70, with approximately equal numbers of males and females) were given either no treatment or a single instillation of 5 mg benzo[*a*]pyrene or an instillation of 50 mg quartz (size, 82% < 2 µm) and 5 mg benzo[*a*]pyrene (Group A) or 50 mg quartz and a later (1 month) instillation of 5 mg benzo[*a*]pyrene (Group B). The rats were observed until death. There were no

Table 3.6 Incidence, numbers and histological types of lung tumours in female Wistar rats after inhalation exposure to quartz and/or Thorotrast

Treatment	Number of rats[a]	Lung tumours				
		Incidence	Total number	Histological type		
		Observed		Bronchiolo-alveolar adenoma	Bronchiolo-alveolar carcinoma	Squamous cell carcinoma
Controls	85	–	–	–	–	–
Low-dose quartz	82	37	62	8	17	37
High-dose quartz	82	43	69	13	26	30
Thorotrast (Tho)	87	3	6	–	5	1
Low-dose quartz + Tho	87	39	68	10	28	30
High-dose quartz + Tho	87	57	98	16	47	35

[a] Without the animals sacrificed 0 and 6 months after the end of inhalation exposure.
From Spiethoff *et al.* (1992)

lung tumours in the untreated rats (0/45), nor in those exposed to benzo[*a*]pyrene alone (0/19). In the combined exposures to benzo[*a*]pyrene and quartz, an increased incidence in lung tumours was observed (Group A, 14/31, 11 squamous cell carcinomas and three papillomas; Group B, 4/18, two papillomas and two carcinomas) (Pylev, 1980). [The Working Group noted the absence of a group exposed to quartz alone.]

(b) Hamster

Groups of 50 male Syrian golden hamsters received weekly intratracheal instillations for 15 weeks in saline of 3 mg benzo[*a*]pyrene or 3 mg ferric oxide or 3 mg ferric oxide plus 3 mg benzo[*a*]pyrene or 1.1 mg Sil-Co-Sil or 1.1 mg Sil-Co-Sil plus 3 mg benzo[*a*]pyrene or 0.7 mg Min-U-Sil or 0.7 mg Min-U-Sil plus 3 mg benzo[*a*]pyrene or 7 mg Min-U-Sil or 7 mg Min-U-Sil plus 3 mg benzo[*a*]pyrene. Fifty male controls received saline alone. Survivors were killed at 92 weeks after exposure. Co-exposures with silica caused an enhancement of the number of respiratory tract tumours induced by benzo[*a*]pyrene (mainly in the bronchus and lung) (Niemeier *et al.*, 1986; Table 3.7).

3.9 Synthesis

Studies of the carcinogenicity of crystalline silica in experimental animal models have shown quartz dust to be a lung carcinogen in rats following inhalation and intratracheal instillation, but not in mice or hamsters. Rats are known to be more sensitive than are mice or hamsters to the induction of lung tumours due to other inhaled poorly soluble particles, such as carbon black (Mauderly *et al.*, 1994).

Quartz-induced lymphoma incidence was also increased in several experiments in rats after intrapleural administration, and in one study in mice after subcutaneous administration. Tridymite- and cristobalite-induced lymphomas were observed in only a single experiment.

Table 3.7 Incidences of respiratory tract tumours in Syrian golden hamsters after intratracheal administration of quartz with or without benzo[*a*]pyrene

Treatment	No. of animals	No. of animals with respiratory tract tumours	No. of respiratory tract tumours[a] by site			Mean latency (wk)
			Larynx	Trachea	Bronchus and lung	
Saline control	48	0	0	0	0	–
BaP	47	22	5	3	32	72.6
Ferric oxide	50	1	1	0	0	62
Ferric oxide + BaP	48	35b,c	5	6	69	70.2
Sil-Co-Sil	50	0	0	0	0	–
Sil-Co-Sil + BaP	50	36b,c	13	13	72	66.5
Min-U-Sil	50	1	0	0	1	68
Min-U-Sil + BaP	50	44b,c	10	2	111	68.5
Min-U-Sil + ferric oxide	49	0	0	0	0	–
Min-U-Sil + ferric oxide + BaP	50	38b,c	10	4	81	66.7

[a] Types of tumours: polyps, adenomas, carcinomas, squamous cell carcinomas, adenosquamous carcinomas, adenocarcinomas, sarcomas.
[b] Statistically significantly higher ($P < 0.00001$; two-tailed Fisher Exact test) compared with the corresponding group not treated with BaP.
[c] Statistically significantly higher ($P < 0.01$; two-tailed Fisher Exact test) compared with the BaP group.
BaP, benzo[*a*]pyrene
From Niemeier *et al.* (1986)

4. Other Relevant Data

4.1 Deposition and biopersistence

The inhalation of crystalline silica is associated with various lung diseases including acute silicosis or lipoproteinosis, chronic nodular silicosis, and lung cancer. Exposure to silica dust may also cause renal and autoimmune diseases (Steenland & Goldsmith, 1995; Stratta *et al.*, 2001; Cooper *et al.*, 2002; Otsuki *et al.*, 2007). In silicotic patients, alveolar macrophages collected by pulmonary lavage contain crystalline silica and at autopsy, elevated levels of quartz are found in the lungs and lymph nodes. Crystalline silica is poorly soluble and biopersistent; even after cessation of exposure, silicosis can progress and is a risk factor for the development of lung cancer (IARC, 1997).

Alveolar macrophages play a key role in silica-related toxicity, and therefore the cytotoxic potency of silica particles determine the degree of silica-related pathogenicity (IARC, 1997; Donaldson & Borm, 1998). The stronger the cytotoxicity of crystalline silica to alveolar macrophages, the higher the intensity of the inflammatory reaction, and the longer the residence time of the particle in the lung (Donaldson & Borm, 1998; Fenoglio *et al.*, 2000a).

Rodent inhalation studies have investigated the relationship between intrinsic particle toxicity, persistent inflammation, altered macrophage-mediated clearance, and biopersistence in the lung (Warheit *et al.*, 2007). Crystalline silica particles induce lung inflammation that persists even after cessation of exposure, with alveolar macrophages having reduced chemotactic responses and phagocytosis. Crystalline silica impairs macrophage-mediated clearance secondary to its cytotoxicity that allows these particles to accumulate and persist in the lungs (IARC, 1997). In humans, it is possible that co-exposure to tobacco smoke and crystalline silica may impair the clearance of these toxic particles (IARC, 2004).

4.2 Mechanisms of carcinogenicity

It is generally accepted that alveolar macrophages and neutrophils play a central role in diseases associated with exposure to crystalline silica (Hamilton *et al.*, 2008). An inflammation-based mechanism as described in IARC (1997) is a likely mechanism responsible for the induction of lung cancer associated with exposure to crystalline silica, although reactive oxygen species can be directly generated by crystalline silica polymorphs themselves, and can be taken up by epithelial cells. For this reason, a direct effect on lung epithelial cells cannot be excluded (Schins, 2002; Fubini & Hubbard, 2003; Knaapen *et al.* 2004).

4.2.1 Physicochemical features of crystalline silica dusts associated with carcinogenicity

The major forms or polymorphs of crystalline silica are the natural minerals quartz, tridymite, cristobalite, coesite, stishovite, and the artifical silica-based zeolites (porosils) (Fenoglio *et al.*, 2000a). Humans have been exposed only to quartz, tridymite, cristobalite, the other forms being very rare. Several amorphous forms of silica exist, some of which were classified in Group 3 (*not classifiable as to their carcinogenicity*) in the previous *IARC Monograph* (IARC, 1997). Also, it has been shown that this cytotoxicity is lowered with lowering hydrophilicity (Fubini *et al.*, 1999), which depends upon the circumstances under which the surface was created. For example, silica in fly ashes or volcanic dusts is generated at high temperatures, and is mostly hydrophobic.

The classification in Group 1 (*carcinogenic to humans*) of some silica polymorphs in the previous *IARC Monograph* (IARC, 1997) was preceded by a preamble indicating that crystalline silica did not show the same carcinogenic potency in all circumstances. Physicochemical features – polymorph characteristics, associated contaminants – may account for the differences found in humans and in experimental studies. Several studies on a large variety of silica samples, aiming to clarify the so-called "variability of quartz hazard" have indicated features and contaminants that modulate the biological responses to silica as well as several surface characteristics that contribute to the carcinogenicity of a crystalline silica particle (Donaldson & Borm, 1998; Fubini, 1998a; Elias *et al.*, 2000; Donaldson *et al.*, 2001). The larger potency of freshly ground dusts (e.g. as in sandblasting) has been confirmed in several studies; Vallyathan *et al.*, 1995), as immediately after cleavage, a large number of surface-active radicals are formed that rapidly decay (Damm & Peukert, 2009). The association with clay or other aluminium-containing compounds inhibits most adverse effects (Duffin *et al.*, 2001; Schins *et al.*, 2002a), iron in traces may enhance the effects but an iron coverage inhibits cytotoxicity and cell transformation (Fubini *et al.*, 2001). One study on a large variety of commercial quartz dusts has shown a spectrum of variability in oxidative stress and inflammogenicity *in vitro* and *in vivo*, which exceeds the differences previously found among different polymorphs (Bruch *et al.*, 2004; Cakmak *et al.*, 2004; Fubini *et al.*, 2004; Seiler *et al.*, 2004). Subtle differences in the level of contaminants appear to determine such variability. New studies *in vitro* and *in vivo* on synthesized nanoparticles of quartz (Warheit *et al.*, 2007) indicate a variability of effects also at the nanoscale. These new data clearly show that more or less pathogenic materials are comprised under the term "crystalline silica dusts." However, most studies, so far, have failed to identify strict criteria to distinguish between potentially more or less hazardous forms of crystalline silica.

The pathogenic potential of quartz seems to be related to its surface properties, and the surface properties may vary depending on the origin of the quartz. The large variability in silica hazard even within quartz particles of the same polymorph may originate from the

grinding procedure, the particle shape, the thermal treatment (determines the hydrophilicity of the particle), and the metal impurities (e.g. aluminium, iron) (Fubini *et al.*, 2004).

The toxicity of silica dust from various sources may be related either to the kind of silica polymorph or to impurities.

The correlation between artificially pure crystalline silicas (porosils) with similar physicochemical properties, but different micromorphology, size and surface area, was investigated (Fenoglio *et al.*, 2000a). Surface area and aspect ratio (elongated crystals with a higher aspect ratio than more isometric crystals) of the particulates tested in a cellular system (mouse monocyte-macrophage tumour cell line) correlate best with inhibition of cell proliferation after 24–72 hours experimental time. From the natural crystalline silicas, only stishovite did not show a cytotoxic effect; all the other natural polymorphs were rather toxic. Stishovite is made up of smooth round particles (Cerrato *et al.*, 1995) whereas quartz, tridymite, and cristobalite consist of particles with very sharp edges caused by grinding (Fubini, 1998a; Fubini *et al.*, 1990, 1999). Stishovite, the only polymorph with silicon in octahedral coordination, has densely packed hydroxyl-silanols on its surface that interact with hydrogen bonds with each other; for this reason, the interaction of silanols with cell membranes, which normally does occur, is dramatically reduced (Cerrato *et al.*, 1995).

Pure silica-zeolites with different particle forms exhibit similar cytotoxicity *in vitro* if compared at equal surface area instead of equal mass. The extent of free radical generation did not show a significant correlation with cytotoxicity in this short-term in-vitro test (Fenoglio *et al.*, 2000a). Free radicals generated by the particle may play a role in later stages of toxicity related to crystalline silica (Ziemann *et al.*, 2009). Both silicon-based surface radicals and iron ions located at the particle surface may be active centres for free radical release in solution (Fubini *et al.*, 2001).

As has already been demonstrated with quartz and cristobalite (Brown & Donaldson, 1996; Bégin *et al.*, 1987), the cytotoxicity of artificially pure silica-zeolites can be decreased by aluminium ions adsorbed onto the particle surface (Fenoglio *et al.*, 2000a). Crystalline silica may occur naturally embedded in clays or may be mixed with other materials in some commercial products. It is possible that these materials may adsorb onto the silica surface, and modify its reactivity. However, the extent of surface coverage and the potency of these modified crystalline silica particles after long-term residence in the lungs have not been systematically assessed.

A quartz sample isolated from bentonite clay obtained from a 100 to 112 million-year-old formation in Wyoming, USA, exhibits a low degree of internal crystal organization, and the surface of this quartz particles are occluded by coatings of the clay. This "quartz isolate" was compared in respect to its toxic potency after intratracheal instillation in rats with the quartz sample DQ12. The "quartz isolate" showed a much lower toxicity than DQ12 quartz, in agreement with the much lower surface reactivity of "quartz isolate" compared to the DQ12 quartz (Creutzenberg *et al.*, 2008; Miles *et al.*, 2008).

4.2.2 Direct genotoxicity and cell transformation

Reactive oxygen species are generated not only at the particle surface of crystalline silica, but also by phagocytic and epithelial cells exposed to quartz particles (Castranova *et al.*, 1991; Deshpande *et al.*, 2002). Oxidants generated by silica particles and by the respiratory burst of silica-activated phagocytic cells may cause cellular and lung injury, including DNA damage. Lung injury may be initiated and amplified by severe inflammation (Donaldson *et al.*, 2001; Castranova, 2004; Knaapen *et al.*, 2004). Various

products (chemotactic factors, cytokines, growth factors) released by activated (and also dying) alveolar macrophages will not only recruit more macrophages as well as polymorphonuclear leukocytes (PMNs) and lymphocytes, but may also affect and activate bronchiolar and alveolar epithelial cells.

Reactive oxygen species can directly induce DNA damage (Knaapen *et al.*, 2002; Schins *et al.*, 2002b), and morphological transformations observed in Syrian hamster embryo (SHE) cells correlate well with the amount of hydroxyl radicals generated (Elias *et al.*, 2000, 2006; Fubini *et al.*, 2001). Neoplastic transformation was observed in in-vitro assays in the absence of secondary inflammatory cells (Hersterberg *et al.*, 1986; Saffiotti & Ahmed, 1995; Elias *et al.*, 2000). There seems to be no correlation between the extent of cytotoxicity as assessed by colony-forming efficiency and transforming potency (SHE test) when various quartz samples were investigated (Elias *et al.*, 2000). In contrast to transforming potency, which was clearly related to hydroxyl radical generation, cytotoxicity was not affected by antioxidants. Partial reduction of transforming potency when deferoxamine-treated quartz was used points to the role of transitional metals, e.g. iron on the particle surface in generating hydroxyl radicals (Fubini *et al.*, 2001). The SHE test used in this study by Fubini *et al.* (2001) and by others is recommended by the Centre for the Validation of Alternative Methods (ECVAM) as an alternative method for investigating the potential carcinogenicity of particulates (Fubini, 1998b). In nude mice injected with these transformed cells, tumours could be initiated (Saffiotti & Ahmed, 1995).

Particle uptake by target cells is also relevant for direct genotoxicity (Schins, 2002). Crystalline silica particles were detected in type II lung epithelial cells (RLE-6TN) *in vitro*; these particles were located also in close proximity to the nuclei and mitochondria, but not within these organelles. An osteosarcoma cell line lacking functional mitochondria was investigated with respect to quartz-related DNA damage with an osteosarcoma cell line with normal mitochondria. Only the cell line with functioning mitochondria showed significantly increased DNA damage after exposure to DQ12 quartz (Li *et al.*, 2007).

The relationship between genotoxic effects (formation of DNA strand breaks) and the uptake of quartz particles was investigated *in vitro* with A549 human lung epithelial cells (Schins *et al.*, 2002a). The percentage of A549 cells containing particles was clearly lower after exposure to quartz coated with polyvinylpynidine-*N*-oxide or aluminum lactate compared to uncoated quartz (DQ12). In this experiment, DNA strand breaks measured (Comet assay) in the exposed cells correlated very well with the number of particles absorbed by the cells. It could also be demonstrated that the generation of reactive oxygen species was closely related to the formation of DNA strand breaks (Schins, 2002). However, in other in-vitro studies using different quartz species, DNA strand breaks in epithelial cells could be observed only at particle concentrations that caused cytotoxicity (Cakmak *et al.*, 2004).

Rats were exposed to crystalline silica for 3 hours and sacrificed at different time points after exposure, and lungs were examined by electron microscopy. The lungs were fixed by vascular perfusion through the right ventricle. In these investigations, silica crystals were found within the cytoplasm of type I lung epithelial cells (Brody *et al.*, 1982). Although type I cells are not the target cell for tumour formation, these data show that the uptake of quartz particles in epithelial lung cells *in vivo* is in principle possible. Other particles including titanium dioxide, carbon black, or metallic particles have occasionally been found in type I lung epithelial cells in rats after inhalation exposure (Anttila, 1986; Anttila *et al.*, 1988; Nolte *et al.*, 1994).

After intratracheal instillation of DQ12 quartz, DNA strand breaks could be observed in lung epithelial cells isolated from quartz-treated rats. This effect was not found when the quartz dust was treated with either polyvinylpyridine-*N*-oxide or aluminium lactate. This finding suggests an important role of the reactive surface of quartz-induced DNA damage *in vivo*. No increase in alkaline phosphatase was found in the bronchiolo-alveolar lavage fluid of quartz-treated rats, and therefore, as alkaline phosphatase is an enzyme specifically present in type II epithelial cells, it can be assumed that there was no obvious cytotoxicity in these lung cells. These data support the current view of the important role of inflammatory cells in quartz-induced genotoxic effects (Knaapen *et al.*, 2002).

4.2.3 Depletion of antioxidant defences

Substantial amounts of ascorbic acid (Fenoglio *et al.*, 2000b) and glutathione (Fenoglio *et al.*, 2003) are consumed in the presence of quartz in cell-free tests via two different surface reactions. Both reactions may deplete antioxidant defences in the lung-lining fluid, thereby enhancing the extent of oxidative damage.

Incubation of murine alveolar MH-S macrophages with quartz particles (80 µg/cm^2) for 24 hours inhibited glucose 6-phosphate dehydrogenase (G6PD)-1 activity by 70%, and the pentose phosphate pathway by 30%. Such effects were accompanied by a 50% decrease in intracellular glutathione. Quartz inhibits G6PD but not other oxidoreductases, and this inhibition is prevented by glutathione, suggesting that silica contributes to oxidative stress also by inhibiting the pentose phosphate pathway, which is critical for the regeneration of reduced glutathione (Polimeni *et al.*, 2008).

4.2.4 Indirect mechanisms

After 13 weeks of inhalation exposure to 3 mg/m^3 crystalline silica (mass median aerodynamic diameter, 1.3 µm) or 50 mg/m^3 amorphous silica (mass median aerodynamic diameter, 0.81 µm), the percentage of PMNs in the lung of the exposed rats was similar after each exposure. However, HPRT mutation frequency of the alveolar epithelial cells was significantly increased only in rats exposed to crystalline silica. Other factors including toxic effects to epithelial cells, solubility, and biopersistence may also be important for the induction of these mutagenic effects (Johnston *et al.*, 2000). A specific finding in rats treated intratracheally with amorphous silica (Aerosil®150, pyrogenic silica with primary particle size of 14 nm) was a severe granulomatous alveolitis which over time progressed to "scar-like" interstitial fibrotic granulomas not seen after crystalline silica exposure in rats (Ernst *et al.*, 2002). Lung tumours were found in rats also after the repeated intratracheal instillation of the same amorphous silica (Kolling *et al.*, 2008).

Toxic mineral dusts, especially crystalline silica, have unique, potent effects on alveolar macrophages that have been postulated to trigger the chain of events leading to chronic lung fibrosis (silicosis) and lung cancer (Hamilton *et al.*, 2008). Macrophages express a variety of cell-surface receptors that bind to mineral dusts leading to phagocytosis, macrophage apoptosis, or macrophage activation. The major macrophage receptor that recognizes and binds inhaled particles as well as unopsonized bacteria is MARCO (Arredouani *et al.*, 2004, 2005). Additional members of the macrophage-scavenger receptor family responsible for binding mineral particles as well as a wide range of other ligands include SR-AI and SR-AII (Murphy *et al.*, 2005). Although SR-AI/II and MARCO bind both toxic and non-toxic particles, only crystalline silica triggers macrophage apoptosis following

binding to these scavenger receptors (Hamilton *et al.*, 2008). Other receptors expressed by macrophages and other target cells in the lung that bind mineral dusts include complement receptor and mannose receptors (Gordon, 2002). The pathological consequences of silica-induced injury to alveolar macrophages followed by apoptosis is impaired alveolar-macrophage-mediated clearance of crystalline silica as discussed in Section 4.1. Lysosomal membrane permeabilization following phagocytosis of crystalline silica particles has been hypothesized to enhance IL-1β secretion (Hornung *et al.*, 2008), and to trigger the release of cathepsin D, leading to mitochondrial damage, and the apoptosis of alveolar macrophages (Thibodeau *et al.*, 2004). Macrophage injury and apoptosis may be responsible for the increased susceptibility of workers exposed to silica to develop autoimmune disease (Pfau *et al.*, 2004; Brown *et al.*, 2005), and pulmonary tuberculosis (IARC, 1997; Huaux, 2007).

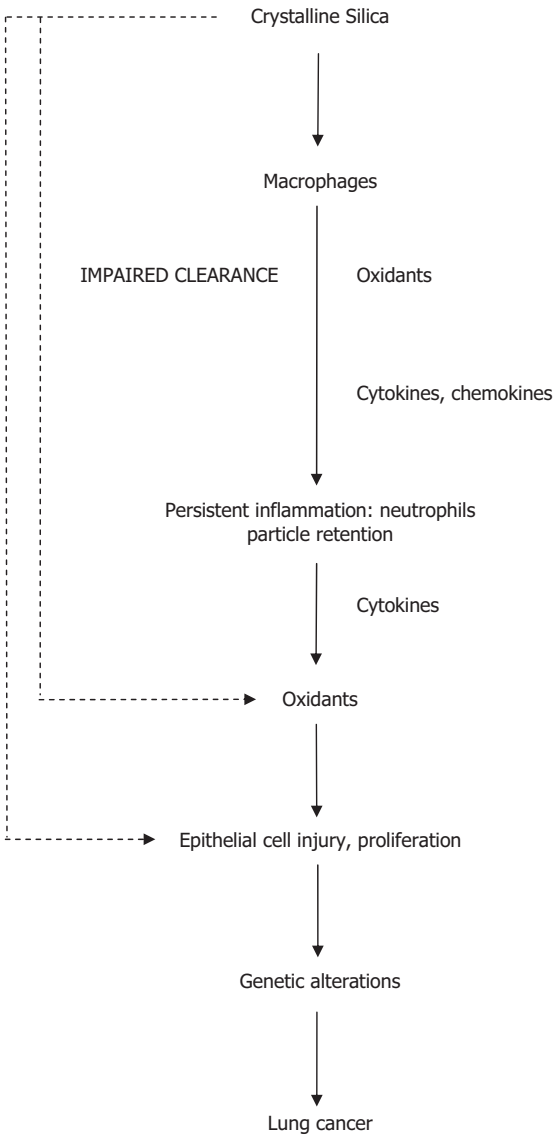
Persistent inflammation triggered by crystalline silica (quartz) has been linked to indirect genotoxicity in lung epithelial cells in rats, see Fig. 4.1 (IARC, 1997). Rats exposed to crystalline silica develop a severe, prolonged inflammatory response characterized by elevated neutrophils, epithelial cell proliferation, and development of lung tumours (Driscoll *et al.*, 1997). These persistent inflammatory and epithelial proliferative responses are less intense in mice and hamsters, and these species do not develop lung tumours following exposure to crystalline silica or other poorly soluble particles (IARC, 1997). There has been considerable discussion of whether the response of rats to inhaled particles is an appropriate model for the exposed response of humans (ILSI, 2000). Comparative histopathological studies of rats and humans exposed to the same particulate stimuli reveal more severe inflammation, alveolar lipoproteinosis, and alveolar epithelial hyperplasia in rats than in humans (Green *et al.*, 2007). These studies suggest that rats are more susceptible to develop persistent lung inflammation in response to particle inhalation than other species (ILSI, 2000).

Chronic exposure of rats to crystalline silica also leads to pulmonary fibrosis (Oberdörster, 1996), and workers with silicosis have an elevated risk of developing lung cancer (Pelucchi *et al.*, 2006). The causal association between chronic inflammation, fibrosis, and lung cancer was reviewed by IARC (2002). These associations provide a biological plausible mechanism between inflammation and the development of fibrosis and/or lung cancer (Balkwill & Mantovani, 2001).

4.3 Molecular pathogenesis of cancer of the lung

Acquired molecular alterations in oncogenes and tumour-suppressor genes characterize the multistage development of lung cancer (Sato *et al.*, 2007). Somatic alterations, such as DNA adducts, develop in the respiratory tract of smokers during the early stages of carcinogenesis (Wiencke *et al.*, 1999). Specific point mutations in in the *K-RAS* oncogene and the *p53* tumour-suppressor gene are considered as biomarkers of exposure to chemical carcinogens in tobacco smoke (Pfeifer *et al.*, 2002). Only one study has investigated the mutational spectrum of these genes that may be used as biomarkers for exposure to crystalline silica. Liu *et al.* (2000) analysed the mutation spectra in the *K-RAS* and *p53* genes in lung cancers that developed in workers with silicosis [smoking status unknown]. In a series of 36 cases, 16 mutations in exons 5, 7 and 8 of the *p53* gene were found. In contrast to non-occupational lung cancers, seven of these mutations clustered in exon 8. Most of the *K-RAS* gene mutations in non-small cell lung carcinomas occur at codon 12. Liu *et al.* (2000) did not detect this mutation in their case series of silicotics. Six mutations were found at codon 15 in exon 1 as well as additional mutations in codons 7, 15, 20, and

Fig. 4.1 Proposed mechanisms for the carcinogenicity of crystalline silica in rats



21. Most of these mutations were G→C transversions in contrast to G→T transversions at codon 12, which are characteristic of non-small cell lung cancers associated with tobacco smoking. If these specific mutations are confirmed in a larger series of lung cancers in silicotics, these could provide early biomarkers for the development of lung cancer in workers exposed to crystalline silica.

In a rat model of silica-induced lung cancer, a low frequency of *p53* gene mutations and no mutations in *K-RAS, N-RAS,* or *c-H-RAS* oncogenes were observed (Blanco *et al.*, 2007). No mutations in oncogenes or tumour-suppressor genes have been directly linked with exposure to crystalline silica.

The epigenetic silencing of the $p16^{INK4a}$ (Belinsky *et al.*, 2002), *CDH13,* and *APC* genes has also been found in a rat model of lung cancer induced by intratracheal instillation of crystalline silica (Blanco *et al.*, 2007). In this rodent model, the increased expression of iNOS

(inducible nitric oxide synthase) was also found in preneoplastic lesions, which is consistent with a role for reactive nitrogen species in silicosis (Porter *et al.*, 2006).

4.4 Species differences and susceptible populations

In rat chronic inhalation studies using crystalline silica or granular, poorly soluble particles, female rats are more susceptible than males to the induction of lung tumours. Overall, rats are susceptible to the induction of lung cancer following the exposure to crystalline silica or granular, poorly soluble particles, but hamsters and mice are more resistant. The mechanistic basis for these sex and species differences is unknown. Mice exposed to crystalline silica by intranasal instillation or subcutaneous injection, as well as rats injected intrapleurally or intraperitoneally develop lymphomas. Following inhalation exposure to crystalline silica, lymphomas have not been observed in any species (see Section 3).

In some workers exposed to crystalline silica, cytokine gene polymorphisms have been linked with silicosis (Yucesoy *et al.*, 2002). Specific polymorphisms in genes encoding in *TNF-α* and *IL-1RA* (interleukin-1 receptor antagonist) have been associated with an increased risk for the development of silicosis (Yucesoy & Luster, 2007). Gene–linkage analyses might reveal additional markers for susceptibility to the development of silicosis and lung cancer in workers exposed to crystalline silica.

4.5 Synthesis

Three mechanisms have been proposed for the carcinogenicity of crystalline silica in rats (Fig. 4.1). First, exposure to crystalline silica impairs alveolar-macrophage-mediated particle clearance thereby increasing persistence of silica in the lungs, which results in macrophage activation, and the sustained release of chemokines and cytokines. In rats, persistent inflammation is characterized by neutrophils that generate oxidants that induce genotoxicity, injury, and proliferation of lung epithelial cells leading to the development of lung cancer. Second, extracellular generation of free radicals by crystalline silica depletes antioxidants in the lung-lining fluid, and induces epithelial cell injury followed by epithelial cell proliferation. Third, crystalline silica particles are taken up by epithelial cells followed by intracellular generation of free radicals that directly induce genotoxicity.

The Working Group considers the first mechanism as the most prominent based on the current experimental data using inhalation or intratracheal instillation in rats, although the other mechanisms cannot be excluded. It is unknown which of these mechanisms occur in humans exposed to crystalline silica dust. The mechanism responsible for the induction of lymphomas in rats and mice following direct injections of crystalline silica dust is unknown.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of crystalline silica in the form of quartz or cristobalite. Crystalline silica in the form of quartz or cristobalite dust causes cancer of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of quartz dust.

There is *limited evidence* in experimental animals for the carcinogenicity of tridymite dust and cristobalite dust.

Crystalline silica in the form of quartz or cristobalite dust is *carcinogenic to humans (Group 1).*

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Exhibit 81

COMPARATIVE EVALUATION OF THE EFFECTS OF TALCUM AND A NEW ABSORBABLE SUBSTITUTE ON SURGICAL GLOVES*

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THE potential hazards of talcum as a lubricant for surgical gloves have long been a matter of concern. The effects of talc within the peritoneal cavity were investigated in 1919 by Hertzler,[1] and subsequently by Haythorn[2] and Antopol.[3] In 1936 Owen[4] conducted an intensive investigation of the subject and suggested that talc be removed completely from the surface of the gloves before operating. Her findings found support in the work of Feinberg,[5] Ramsey,[6,7] Byron and Welch,[8] Weed and Groves[9] and German.[10]

Lichtman, McDonald, Dixon and Mann,[11] in the course of an extensive study of birefringence in tissues and an investigation of changes in tissues in human beings and laboratory animals produced by doubly refractile foreign bodies made visible by polarized light, offered incontrovertible evidence of the local irritant action of talcum. They found that the characteristic reaction in human tissues consisted of the formation of pseudotubercles and that crystals of talc implanted in artificially created fistulas in dogs caused a persistence of the fistulas. Their findings lent considerable emphasis to the need for an adequate substitute for this irritating substance.

Seelig[12,13] and Seelig, Verda and Kidd[14,15] who have repeatedly warned of the dangers of talc in operative wounds, offered potassium bitartrate and, subsequently, an experimentally produced formaldehyde-treated starch as possible substitutes. Both of these substances have been found to be unsatisfactory for a variety of reasons.

In view of the grave dangers attendant upon the use of talcum, a great deal of effort has been devoted to the search for a satisfactory substitute. Of many compounds tested by the authors and others, in the laboratory and clinically, one has proved so promising as to warrant its introduction for widespread clinical use under practical operating room conditions. This substance consists of a mixture of amylose and amylopectin, derived from corn starch, which has been treated by physical and chemical means to improve its lubricating value and to prevent gelatinization when autoclaved; a small amount (1 per cent) of magnesium oxide is included for the purpose of further improving the flow properties of the mixture. For the sake of convenience this preparation has been designated Biosorb absorbable powder.†

Lee and Lehman[16] found that this material possessed excellent physical qualities of flow and fineness which were largely unaffected by autoclaving and that it was completely absorbable from the peritoneum without inflammatory reaction and without the formation of adhesions. Their clinical experience with the powder, as well as that of Walkling and Lindenmuth,[17] proved eminently satisfactory. MacQuiddy and Tollman[18] who investigated the anaphylactogenic properties of biosorb powder were unable to find any sensitivity to this modified starch in any of the humans tested, nor was it possible to produce a state of sensitivity in animals when this

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* From the Laboratories of Johnson & Johnson, New Brunswick, New Jersey.

substance was used as the anaphylactogen. Their findings further supported the observations of others that this material is non-irritating, is absorbed readily from the peritoneal cavity and appears to be an entirely satisfactory replacement for talcum powder for surgical purposes.

In view of the potential importance of this new powder and the fact that some of the previously offered substitutes (notably potassium bitartrate) have proven inadequate, in part because of their deleterious effects on rubber gloves, it was deemed of interest to conduct a detailed comparison of the relative effects of talcum powder and Biosorb powder on the rates of deterioration of several brands of gloves prepared with these substances and subjected to repeated autoclavings.

It is, of course, well known that steam sterilization alone produces considerable deterioration of surgical rubber gloves, but the question of importance to be determined was whether the rate of this deterioration is accelerated when the gloves are treated with Biosorb instead of talc.

Surgical gloves were purchased from six manufacturers; they comprised several types, including those fabricated from neoprene, white latex and brown latex. The gloves were divided equally into two sets, those in one set being treated in all subsequent procedures with talcum powder and those in the other with Biosorb powder. The preparation of the gloves before each autoclaving followed standard hospital practice as closely as possible. The gloves were washed thoroughly with soap and water, air dried and dusted inside and out with the lubricant powder. A pledget of powder-impregnated gauze was then placed in the cuff of the right hand glove, each pair placed in linen glove folders and autoclaved at 15 pounds pressure (240°F.) for thirty minutes.

After each sterilization the gloves were examined for flaws during moderate distension, washed free of powder and allowed to air dry for a period of twenty-four hours; at the end of this period they again were prepared, respectively, with talcum or Biosorb and autoclaved, the cycle being repeated the requisite number of times.

Tensile strength measurements were made on each set of gloves before sterilization and after groups of three, six and nine consecutive autoclavings. While a number of possible methods of determining the degree of deterioration suggested themselves, it was thought that measurement of the tensile strength probably was the most accurate quantitatively with the facilities available to us. This factor was correlated with the ability of the glove to withstand the stress and strain of application under clinical conditions as a qualitative measure of the point of failure under conditions of practicable use. It was found that, in general, when the tensile strength became less than 1.5 pounds the likelihood of tearing during the act of putting on the gloves, or during subsequent manipulations, was great.

The tensile strengths were determined on sections measuring 0.25 by 2.0 inches (.635 by 5.08 cm.) cut from the gloves with a special die. A Scott tensile strength tester was used with the jaws set at 0.5 inch (1.27 cm.) apart and the results were recorded as pounds pull at the moment of rupture. Since the material used in the manufacture of surgical gloves is not of uniform thickness, thickness measurements were made on each section tested, using a standard thickness micrometer of the type used in determining the diameter of catgut. These determinations were correlated with the tensile strength results, which thus are corrected for differences in thickness of the samples tested; 0.01 inch (0.25 mm.) was taken as the standard thickness. The results are summarized in Table 1. Each result listed in the table represents the average of five determinations per glove. We wish to emphasize that we are not offering these data as absolute values indicating true tensile strengths. The principal interest is in determining relative effects; since each determination was obtained in an identical

TABLE I

COMPARATIVE TENSILE STRENGTH OF SURGEON'S RUBBER GLOVES WHEN TREATED WITH TALCUM AND BIOSORB ABSORBABLE POWDER

POUNDS PULL OF RUBBER SECTION 0.25 BY 0.01 INCH

Brand	I			II			III	IV	V	VI	Grand Averages
Type	White Latex	Brown Latex	Brown Latex	White Latex	Brown Latex	Neoprene	Brown Latex	Brown Latex	White Latex	White Latex	
Control (without any powder and not sterilized)	4.9	2.6	4.4	2.6	4.9	6.6	4.3	2.4	5.2	5.0	
	5.8	4.3	4.3	5.0	5.1	5.2	4.1	2.9	4.5	5.2	
	4.0	3.8	5.2	4.9	5.5	2.8	4.4	2.6	5.1	5.2	
	3.6	2.8	5.1	5.8	3.5	3.0	4.2	2.6	5.0	4.1	
	5.4	2.8	4.2	3.3	4.7	5.6	4.8	2.0	5.3	5.4	
$\bar{X}$	4.9	3.3	4.6	4.3	4.7	4.6	4.4	2.4	5.0	5.0	$\bar{\bar{X}}$ 4.32
Biosorb absorbable powder 3 sterilizations	4.8	1.9	2.9	3.3	3.6	1.7	3.8	1.8	3.0	1.4	
	3.1	2.1	3.1	3.4	4.0	2.1	3.6	2.0	4.0	1.4	
	2.9	2.2	3.1	4.0	4.5	5.1	2.2	1.6	2.5*	1.2	
	4.0	2.4	4.0	3.7	5.5	2.2	3.6	2.1	3.5	1.5	
	3.2	2.0	3.9	3.7	4.2	8.2	3.4	0.8	2.7	1.2	
$\bar{X}$	3.6	2.1	3.4	3.6	4.4	3.9	3.3	1.7	3.1	1.3	$\bar{\bar{X}}$ 3.04
Talcum powder 3 sterilizations	3.6	2.2	3.7	3.6	5.4	4.6	3.1	1.8	2.8	1.2	
	3.2	2.0	3.2	3.7	5.2	4.5	2.7	1.7	1.3	1.2	
	3.2	2.2	2.5	4.3	3.5	3.6	4.1	1.5	1.8	1.3	
	3.4	1.8	2.8	4.1	4.8	6.1	3.2	1.2	1.9	1.2	
	2.6	1.8	3.7	3.7	4.6	2.2	3.2	0.9	2.5	1.0	
$\bar{X}$	3.2	2.0	3.2	3.9	4.7	4.2	3.3	1.4	2.1	1.2	$\bar{\bar{X}}$ 2.92
Biosorb absorbable powder sterilizations	1.8	1.7	2.6	2.7	3.8	5.2	1.9	1.1	2.6	0.8	
	2.1	1.8	2.6	1.6	1.8	3.8	2.2	0.6	2.4	0.6	
	2.2	1.3	2.9	2.7	3.1	1.8	3.8	0.9	1.9	0.6	
	2.2	1.6	2.6	2.5	2.4	2.2	2.4	0.5	2.4	0.6	
	2.5	1.3	3.1	2.5	3.1	5.1	3.0	0.7	2.4	0.5	
$\bar{X}$	2.2	1.5	2.8	2.4	2.8	3.6	2.7	0.8	2.3	0.6	$\bar{\bar{X}}$ 2.17
Talcum powder 6 sterilizations	1.9	1.6	2.0	2.9	3.6	4.6	3.3	0.9	2.0	0.8	
	1.8	1.2	3.0	2.0	2.6	2.5	2.0	0.9	1.1	0.7	
	1.9	1.5	2.3	2.8	2.3	1.8	1.9	0.7	1.7	0.7	
	2.7	1.2	2.4	2.3	2.6	2.4	1.9	1.0	2.0	0.6	
	2.5	1.2	1.9	2.0	2.6	2.2	2.4	0.8	2.3	0.7	
$\bar{X}$	2.2	1.3	2.3	2.4	2.7	2.7	2.3	0.9	1.8	0.7	$\bar{\bar{X}}$ 1.93
Biosorb absorbable powder 9 sterilizations	1.5	1.1	1.7	1.6	2.7	5.8	2.1	0.6	2.3	0.5	
	2.7	1.0	1.6	1.8	3.0	3.2	1.7	0.6	1.9	0.5	
	1.6	1.6	2.0	2.3	2.8	6.5	2.7	1.1	1.4	0.5	
	1.6	1.1	2.1	2.1	2.8	4.9	1.7	0.6	1.5	0.6	
	2.2	1.1	1.4	1.8	4.2	2.8	3.2	0.5	1.4	0.4	
$\bar{X}$	1.9	1.2	1.8	1.9	3.1	4.6	2.3	0.7	1.7	0.5	$\bar{\bar{X}}$ 1.97
Talcum powder 9 sterilizations	1.6	1.7	1.6	2.3	3.1	7.4	1.8	0.5	1.2	0.5	
	1.6	1.2	1.8	2.4	2.8	5.0	2.1	0.7	1.0	0.3	
	1.7	.7	1.6	2.7	2.7	2.9	1.6	0.6	1.5	0.4	
	1.8	.9	1.7	2.2	2.7	3.8	1.6	0.8	0.6	0.2	
	1.7	.9	1.6	1.7	2.8	2.1	1.8	0.5	1.0	0.3	
$\bar{X}$	1.7	1.1	1.7	2.3	2.8	4.2	1.8	0.6	1.1	0.3	$\bar{\bar{X}}$ 1.76

$\bar{X}$ = Average of five tests.

manner, the various data are comparable on a relative basis. The respective brands are designated by Roman numerals.

An analysis* of the data in Table I indicates considerable variability among the tensile strengths of surgeons' gloves of various types and between gloves of a given manufacturer, even before preparatory dusting and sterilization. Initial observation without the benefit of such an

* We are indebted to Mr. E. H. MacNiece for a statistical analysis of our data.

analysis might create flash impressions as to the superiority of either lubricant but analysis shows that the gloves treated with the absorbable powder averaged 4 per cent stronger than gloves treated with talc, after three sterilizations. After six and nine sterilizations, Biosorb powder-treated gloves averaged about 12 per cent stronger than those treated with talc.

To determine the significance of the difference between the effects of the two lubricants on the degradation of tensile strength, a "t" test was made. The "t" value between the two lubricants computed on the thirty, paired, averaged, tensile strengths of groups of five samples is 3.16 with 29 degrees of freedom, indicating a highly significant difference in favor of the Biosorb powder. The interaction between the types of lubricant and types of fabric is insignificant, indicating that Biosorb powder retards the degrading effect of steam sterilization on the tensile strength of all three types of rubber.

With the brand of glove manifesting the least rapid rate of deterioration the fatigue point was not reached until the gloves had been subjected to nine autoclavings, regardless of whether talc or Biosorb powder was used as the lubricant. This fact is of considerable importance from the standpoint of operating room administration since there is considerable variation among all types of gloves in the inherent susceptibility of the material to autoclaving and adverse rates of deterioration are by no means necessarily attributable to the lubricating powder used in preparing the gloves.

This study statistically indicates a betterment in glove life and reliability by the use of Biosorb powder and it is clear on empirical grounds that this powder does not produce a more rapid rate of deterioration in comparison with that which occurs during steam sterilization when talc is used as the dusting agent. Thus, the factor of adverse effect upon rubber gloves is not encountered in avoiding the obvious hazards of talcum powder when this absorbable modified starch is used as the replacing agent.

SUMMARY AND CONCLUSIONS

1. A series of rubber gloves obtained from six manufacturers were divided into two sets and subjected to a comparative series of autoclavings after treatment, respectively, with talcum and a new absorbable starch powder.

2. The respective rates of deterioration were compared; it was found that when the absorbable powder was used the degradation in the tensile strength of the gloves produced by autoclaving occurred less rapidly upon repeated sterilizations than when talcum was used as the dusting agent.

3. The effect of this absorbable powder on rubber gloves does not constitute a drawback to its use as a replacement for talcum powder as a lubricating agent.

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Certain living tissues will grow in a medium consisting of Tyrode's solution, buffered salt solution, heparinized plasma and embryonic extract. Neither the plasma nor the embryonic extract need come from the same species of animal.

From "Principles and Practice of Surgery" by W. Wayne Babcock (Lea & Febiger).

Exhibit 82

to be informed promptly and effectively of important new knowledge regarding nutritional and health benefits of food. Third, these amendments to this health claim will ensure that scientifically sound nutritional and health information regarding the benefits of fruit and vegetable intake and reduction of CHD risk can be provided to consumers as soon as possible. The past few editions of the DGA have been moving away from a focus on total fat and have instead communicated to consumers the need to focus on type of fat consumed instead of total amount of fat. Recent editions of the DGA have also encouraged increased intake of fruits and vegetables for a healthful diet. Prompt issuance of an interim final rule that reflects the current recommendations is necessary for consumers to be able to have the most current information on nutrition and diet. Consumers will be better able to construct healthful diets if they have prompt access to information that is consistent with the current recommendations on fat content and on consumption of fruits and vegetables. Therefore, we are using the authority in section 403(r)(7)(A) of the FD&C Act to issue an interim final rule amending the general requirements for the health claim for dietary saturated fat and cholesterol and risk of CHD and to make the interim final rule effective immediately.

This regulation is effective upon publication in the **Federal Register**. We invite public comment on this interim final rule. We will consider modifications to this interim final rule based on comments made during the comment period. We will address comments and confirm or amend the interim final rule in a final rule.

X. References

The following references are on display in the Division of Dockets Management (see **ADDRESSES**) and are available for viewing by interested persons between 9 a.m. and 4 p.m., Monday through Friday; they are also available electronically at *http://www.regulations.gov.* FDA has verified the Web site addresses, as of the date this document publishes in the **Federal Register,** but Web sites are subject to change over time.

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7. U.S. Department of Health and Human Services and U.S. Department of Agriculture. ''Dietary Guidelines for Americans, 2005. 6th Edition,'' 2005. Available at *http://health.gov/dietaryguidelines/dga2005/document/default.htm.*

8. Institute of Medicine (IOM) of the National Academies. ''Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).'' Chapter 8, ''Dietary Fats: Total Fat and Fatty Acids,'' 2002.

9. FDA/CFSAN, Food Labeling: Health Claims; Dietary Saturated Fat and Cholesterol and Risk of Coronary Heart Disease, Regulatory Impact Analysis, FDA–2013–P–0047.

List of Subjects in 21 CFR Part 101

Food labeling, Nutrition, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 101 is amended as follows:

PART 101—FOOD LABELING

■ 1. The authority citation for part 101 continues to read as follows:

Authority: 15 U.S.C. 1453, 1454, 1455; 21 U.S.C. 321, 331, 342, 343, 348, 371; 42 U.S.C. 243, 264, 271.

■ 2. Section 101.75 is amended by revising paragraphs (c)(1) and (c)(2)(ii) to read as follows:

§ 101.75 Health claims: dietary saturated fat and cholesterol and risk of coronary heart disease.

* * * * *

(c) * * *

(1) All requirements set forth in § 101.14 shall be met, except § 101.14(e)(6) with respect to a raw fruit or vegetable.

(2) * * *

(ii) *Nature of the food.* (A) The food shall meet all of the nutrient content requirements of § 101.62 for a ''low saturated fat'' and ''low cholesterol'' food.

(B) The food shall meet the nutrient content requirements of § 101.62 for a ''low fat'' food, unless it is a raw fruit or vegetable; except that fish and game meats (*i.e.,* deer, bison, rabbit, quail, wild turkey, geese, and ostrich) may meet the requirements for ''extra lean'' in § 101.62.

* * * * *

Dated: December 9, 2016.

Leslie Kux,

Associate Commissioner for Policy.

[FR Doc. 2016–29997 Filed 12–16–16; 8:45 am]

BILLING CODE 4164–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 878, 880, and 895

[Docket No. FDA–2015–N–5017]

RIN 0910–AH02

Banned Devices; Powdered Surgeon's Gloves, Powdered Patient Examination Gloves, and Absorbable Powder for Lubricating a Surgeon's Glove

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA or Agency) has determined that Powdered Surgeon's Gloves, Powdered Patient Examination Gloves, and Absorbable Powder for Lubricating a Surgeon's Glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling. Consequently, FDA is banning these devices.

DATES: This rule is effective on January 18, 2017.

ADDRESSES: For access to the docket to read background documents or comments received, go to *https://www.regulations.gov* and insert the docket number found in brackets in the

heading of this final rule into the ''Search'' box and follow the prompts, and/or go to the Division of Dockets Management, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852.

FOR FURTHER INFORMATION CONTACT: Michael J. Ryan, Center for Devices and Radiological Health, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 66, Rm. 1615, Silver Spring, MD 20993, 301–796–6283, email: *michael.ryan@fda.hhs.gov*.

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I. Executive Summary

A. Purpose and Coverage of the Final Rule

Medical gloves play a significant role in the protection of both patients and health care personnel in the United States. Health care personnel rely on medical gloves as barriers against transmission of infectious diseases and contaminants when conducting surgery, as well as when conducting more limited interactions with patients. Various types of powder have been used to lubricate gloves so that wearers could don the gloves more easily. However, the use of powder on medical gloves presents numerous risks to patients and health care workers, including inflammation, granulomas, and respiratory allergic reactions.

A thorough review of all currently available information supports FDA's conclusion that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove should be banned. FDA has concluded that the risks posed by powdered gloves, including health care worker and patient sensitization to natural rubber latex (NRL) allergens, surgical complications related to peritoneal adhesions, and other adverse health events not necessarily related to surgery, such as inflammatory responses to glove powder, are important, material, and significant in relation to the benefit to public health from their continued marketing. FDA has carefully evaluated the risks and benefits of powdered gloves and the risks and benefits of the state of the art, which includes viable non-powdered alternatives that do not carry any of the risks associated with glove powder, and has determined that the risk of illness or injury posed by powdered gloves is unreasonable and substantial. Further, FDA believes that this ban would likely have minimal economic and shortage impact on the health care industry. Thus, a transition to alternatives in the marketplace should not result in any detriment to public health.

This rule applies to powdered patient examination gloves, powdered surgeon's gloves, and absorbable powder for lubricating a surgeon's glove. This includes all powdered medical gloves except powdered radiographic protection gloves. Because we are not aware of any powdered radiographic protection gloves that are currently on the market, FDA lacks the evidence to determine whether the banning standard would be met for this particular device. The ban does not apply to powder used in the manufacturing process (*e.g.,* former-release powder) of non-powdered gloves, where that powder is not intended to be part of the final finished glove. Finished non-powdered gloves are expected to include no more than trace amounts of residual powder from these processes, and the Agency encourages manufacturers to ensure finished non-powdered gloves have as little powder as possible. In our 2008 Medical Glove Guidance Manual (Ref. 1), we recommended that non-powdered gloves have no more than 2 milligrams (mg) of residual powder and debris per glove, as determined by the Association for Testing and Materials (ASTM) D6124 test method (Ref. 2). The Agency continues to believe this amount is an appropriate maximum level of residual powder. The ban also does not apply to powder intended for use in or on other medical devices, such as condoms. FDA has not seen evidence that powder intended for use in or on other medical devices, such as condoms, presents the same public health risks as that on powdered medical gloves.

B. Summary of the Major Provisions of the Final Rule

In this final rule, FDA is banning the following devices: (1) Powdered surgeon's gloves, (2) powdered patient examination gloves, and (3) absorbable powder for lubricating a surgeon's glove. Because the classification regulations for these device types do not distinguish between powdered and non-powdered versions, FDA is amending the descriptions of these devices in the regulations to specify that the regulations for patient examination and surgeon's gloves will apply only to non-powdered gloves while the powdered version of each type of glove will be added to the listing of banned devices in the regulations.

Many comments requested that FDA revise the scope of the ban to include all NRL gloves. Many comments from industry requested that the proposed effective date be extended beyond 30 days after the date of publication of the final rule. Of the comments that do not support the ban, commenters noted the need for powdered gloves to aid in donning gloves and tactile sense and the reduced risks associated with current powdered gloves that have less powder. The remaining comments are not clearly in support or opposition to the proposal.

C. Legal Authority

Powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove are defined as devices under section 201(h) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 321(h)). Section 516 of the FD&C Act (21 U.S.C. 360f) authorizes FDA to ban a device if it finds, on the basis of all available data and information, that the device presents substantial deception or unreasonable and substantial risks of illness or injury, which cannot be corrected by labeling or a change in labeling. This rule amends 21 CFR 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104. FDA's legal authority to modify §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104 arises from the device and general administrative provisions of the FD&C Act (21 U.S.C. 352, 360f, 360h, 360i, and 371).

D. Costs and Benefits

The final rule is expected to provide a positive net benefit (estimated benefits minus estimated costs) to society. Banning powdered glove products is not expected to impose any costs to society, but is expected to reduce the number of adverse events associated with using powdered gloves. The primary public health benefit from adoption of the rule would be the value of the reduction in adverse events associated with using powdered gloves. The Agency estimates maximum total annual net benefits to range between $26.8 million and $31.8 million.

II. Background

A. Need for the Regulation/History of the Rulemaking

On March 22, 2016, FDA issued a proposed rule to ban powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove (81 FR 15173). Section 516(a)(1) of the FD&C Act authorizes FDA to ban a device intended for human use by regulation if it finds, on the basis of all available data and information, that such a device ''presents substantial deception or an unreasonable and substantial risk of illness or injury.'' For a more detailed discussion of the banning standard, we refer you to the preamble of the proposed rule. FDA issued the proposed regulation because it determined that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling.

The preamble to the proposed rule describes the history of powdered gloves and the citizen petitions received by the Agency that request a ban on powdered gloves. We refer readers to that preamble for information about the development of the proposed rule. The level and types of risk presented by powdered gloves varies depending on the composition and intended use of the glove. In aggregate, the risks of powdered gloves include severe airway inflammation, hypersensitivity reactions, allergic reactions (including asthma), allergic rhinitis, conjunctivitis, dyspnea, as well as granuloma and adhesion formation when exposed to internal tissue. We refer readers to the preamble of the proposed rule for details on the level and types of risks presented by powdered gloves. The benefits of powdered gloves appear to only include greater ease of donning and doffing, decreased tackiness, and a degree of added comfort, which FDA believes are nominal when compared to the risks posed by these devices.

The state of the art of both surgeon's and patient examination gloves includes non-powdered alternatives that provide similar performance as the various powdered glove types do. That is, there are many non-powdered gloves available that have the same level of protection, dexterity, and performance. Thus, based on a careful evaluation of the risks and benefits of powdered gloves and the risks and benefits of the current state of the art, which includes readily available alternatives that carry none of the risks posed by powdered gloves, FDA has determined that the standard to ban powdered gloves has been met, and that it is appropriate to issue this ban.

Finally, as discussed in the proposed rule, FDA also determined the ban should apply to devices already in commercial distribution and devices already sold to the ultimate user, as well as to devices that would be sold or distributed in the future (see 21 CFR 895.21(d)(7)). This means that powdered gloves currently being used in the marketplace would be subject to this ban and adulterated under section 501(g) of the FD&C Act (21 U.S.C. 351(g)), and thus subject to enforcement action.

B. Summary of Comments to the Proposed Rule

The Agency requested public comments on the proposed rule, and the comment period closed on June 20, 2016. The Agency received approximately 100 comment letters on the proposed rule by the close of the comment period, each containing one or more comments on one or more issues. We received comments from a cross-section of patients and consumers, medical professionals, device manufacturers, and professional and trade associations. A majority of the comments supported the objectives of the rule in whole or in part, while a minority of the comments opposed the objectives of the rule. Some comments suggested changes to specific elements of the proposed rule or requested clarification of matters discussed in the proposed rule. See Section IV for the description of comments on the proposed rule and FDA's responses.

C. General Overview of the Final Rule

FDA published a proposed rule to ban powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove, because FDA determined that these devices present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling (81 FR 15173).

In this final rule, FDA is banning the following devices: (1) Powdered surgeon's gloves (21 CFR 878.4460), (2) powdered patient examination gloves (21 CFR 880.6250), and (3) absorbable powder for lubricating a surgeon's glove (21 CFR 878.4480). Because the classification regulations for these device types do not distinguish between powdered and non-powdered versions, FDA is amending the descriptions of these devices in the regulations to specify that the regulations for surgeon's gloves (21 CFR 878.4460) and patient examination gloves (21 CFR 880.6250) will apply only to non-powdered gloves while the powdered version of each type of glove will be added to 21 CFR part 895, subpart B—Listing of Banned Devices.

D. Clarifying Changes to the Rule

While FDA believes that the preamble to the proposed rule was clear that the proposed ban would apply to all powdered surgeon's gloves and all powdered patient examination gloves, in reviewing the terminology used in the proposed additions to 21 CFR part 895, FDA determined that term ''synthetic latex'' would not cover every type of non-NRL material that is used to manufacture powdered gloves. It was not FDA's intent to limit the ban to only powdered NRL and powdered synthetic latex gloves, and we believe that this intent was clear from the content of the preamble to the proposed rule, which stated that the ban ''would apply to all powdered gloves except powdered radiographic protection gloves.'' As such, FDA has now revised the identification in this final rule to clarify that the ban applies to all powdered surgeon's gloves and powdered patient examination gloves without reference to the type of material from which they are made. Additionally, the identification of non-powdered surgeon's gloves and non-powdered patient examination gloves is also being revised to remove reference to material.

III. Legal Authority

Powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove are defined as medical devices under section 201(h) of the FD&C Act (21 U.S.C. 321). Section 516 of the FD&C Act (21 U.S.C. 360f) authorizes FDA to ban a device if it finds, on the basis of all available data and information, that the device

presents substantial deception or unreasonable and substantial risks of illness or injury, which cannot be corrected by labeling or a change in labeling. This rule amends §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104. FDA's legal authority to modify §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104 arises from the device and general administrative provisions of the FD&C Act (21 U.S.C. 352, 360f, 360h, 360i, and 371).

IV. Comments on the Proposed Rule and FDA's Responses

A. Introduction

We received approximately 100 comment letters on the proposed rule by the close of the comment period, each containing one or more comments on one or more issues. We received comments from a cross-section of patients and consumers, medical professionals, device manufacturers, and professional and trade associations. A majority of the comments supported the objectives of the rule in whole or in part, while a minority of the comments opposed the objectives of the rule. Some comments suggested changes to specific elements of the proposed rule or requested clarification of matters discussed in the proposed rule.

We describe and respond to the comments in section IV.B through E. We have numbered each comment to help distinguish between different comments. We have grouped similar comments together under the same number, and, in some cases, we have separated different issues discussed in the same comment and designated them as distinct comments for purposes of our responses. The number assigned to each comment or comment topic is purely for organizational purposes and does not signify the comment's value or importance or the order in which comments were received.

B. Description of General Comments and FDA Response

Many comments made general remarks supporting or opposing the proposed rule without focusing on a particular proposed provision. In the following paragraphs, we discuss and respond to such general comments.

(Comment 1) Many comments support the proposed ban on powdered patient examination gloves and powdered surgeon's gloves. These comments from individual consumers, health care professionals, academia, and industry highlight several risks of the continued use of powdered gloves, including, among others, allergic reactions, post-operative adhesions, and delayed wound healing.

(Response 1) FDA agrees with these comments. After further review of all available information and the comments submitted to the proposed rule, FDA has concluded that the public's exposure to the risks of powdered gloves is unreasonable and substantial in relation to the nominal public health benefit derived from the continued marketing of these devices, especially when considering the benefits and risks posed by readily available alternative devices. Therefore, FDA has determined that the standard for a ban on these devices has been met.

C. Description of Comments That Oppose the Regulation and FDA Response

FDA received some comments that oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves for various reasons. We address each of these reasons for opposition in this section. After reviewing these comments, FDA has determined that the standard to ban powdered gloves has been met, and that it is appropriate to issue this ban. We are finalizing the ban with only clarifying changes.

(Comment 2) Comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because of difficulty donning or doffing non-powdered gloves. Two commenters specifically discuss hyperhidrosis with claims that it can add to the difficulty donning and doffing non-powdered gloves. One commenter has asserted that double-gloving is more difficult when using non-powdered gloves.

(Response 2) As described in the preamble of the proposed rule, we have concluded that the benefit of ease of donning or doffing powdered gloves is generally nominal (Ref. 3) in comparison to the risks posed by the continued marketing of powdered gloves, which, among others, include severe airway inflammation, hypersensitivity reactions, and allergic reactions (including asthma). Also, as noted in the proposed rule, a study of various brands of powdered and non-powdered NRL gloves by Cote et al. found that there are non-powdered latex gloves that are easily donned with wet or dry hands with relatively low force compared to the forces required to don powdered latex examination gloves (Ref. 3). Thus, FDA has considered ease of donning and doffing as a benefit as it applies within the banning standard, and has determined that the standard is met.

(Comment 3) Comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because of difficulty donning non-powdered gloves, leading to greater propensity of non-powdered gloves to tear. Some of these comments express concern that the reduced ability to separate the opening of a non-powdered glove or the greater propensity of non-powdered gloves to tear could potentially lead to a higher degree of contamination and post-procedure infections.

(Response 3) FDA disagrees with the assertion that non-powdered gloves have a higher propensity to tear and thus disagrees that use of non-powdered gloves presents a greater risk of contamination, post-procedure infections, or exposure of the user to blood. FDA does not believe there is compelling evidence to support the assertion that non-powdered gloves have a higher propensity to tear. Korniewicz, et al., determined that the presence of powder did not affect the durability of gloves or enhance glove donning (Ref. 4). Although Kerr, et al., identified a statistically significant difference in the durability of non-powdered vinyl gloves compared to powdered vinyl gloves, this difference may be attributed to glove type, manufacturer, and the fingernail length of users rather than the presence or absence of powder (Ref. 5). This study also found that vinyl gloves in general are less durable and have a greater propensity to tear compared to nitrile, neoprene, and latex gloves. Furthermore, as discussed in the response to comment 4, several studies have found that alternatives to non-powdered NRL gloves, such as nitrile and neoprene gloves, offer the same level of protection against contamination and exposure to blood as powdered NRL gloves (Refs. 5, 6, 7, 8, 9, and 10). Therefore, FDA has determined that suitable alternatives to powdered gloves are readily available in the marketplace.

(Comment 4) Commenters oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because the fit of powdered gloves is more comfortable than non-powdered gloves. Some of these comments assert that the reduced fit of non-powdered gloves inhibits the tactile sensation necessary to perform medical procedures.

(Response 4) FDA disagrees with the assertion that non-powdered gloves inhibit the tactile sensation necessary to perform medical procedures. The ban does not include non-powdered NRL gloves, which offer the same

performance characteristics of powdered NRL gloves, and several studies have found that alternatives, such as nitrile and neoprene gloves, offer the same level of protection, dexterity, and performance as NRL gloves (Refs. 5, 6, 7, 8, 9, and 10). Furthermore, the numerous risks posed by the continued marketing of powdered gloves outweigh the benefit of whatever additional level of comfort is provided from using powdered gloves instead of the non-powdered alternatives that carry none of these risks.

(Comment 5) Some comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, citing a lack of scientific evidence that gloves with reduced powder content, as those in use today, have the same risks as previously used gloves that had higher powder content.

(Response 5) FDA agrees that the maximum residual level of powder on powdered gloves is less than earlier types of powdered gloves. Historically, powdered medical gloves contained powder levels ranging from 50 to over 400 mg of powder per glove. Effective in 2002, the ASTM International recommended limits on powder levels is 15 mg per square decimeter for surgical gloves (ASTM D3577–2001) (Ref. 11) and 10 mg per square decimeter for patient examination gloves (ASTM D3578) (Ref. 12). As a result, FDA believes that gloves in use after 2002 follow these recommended limits and generally have lower powder content than earlier types of powdered gloves. Even so, several studies indicate that gloves with reduced powder levels continue to present unreasonable and substantial risks to patients and health care workers. For instance, a study conducted on the incidence of skin reactions for Greek endodontists from 2006 to 2012 found that glove powder accounted for the majority of skin reactions, and the replacement of powdered NRL gloves with non-powdered gloves resolved the majority of the adverse reactions (Ref. 13). Similarly, the risks of powdered gloves persist in non-clinical studies using gloves with reduced powder content, as demonstrated by the 2013 finding that surgeries performed with powdered gloves increased the number, density, and fibrotic properties of peritoneal adhesions in rats compared with surgeries performed with non-powdered gloves (Ref. 14). Also, the reduction in cases of NRL-induced occupational contact urticaria coincided with French hospitals transitioning to non-powdered gloves after 2004–2005 (Ref. 13). Finally, FDA is not aware of any report in the literature that supports the assertion that currently marketed powdered gloves with lower powder content reduce the risks presented by powdered gloves (Ref. 15). In summary, FDA concludes that the risks of powder continue to be unreasonable and substantial for currently marketed powdered gloves despite lower powder content than previous generations of powdered gloves.

(Comment 6) Two comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the commenters believe a warning on the risks of powdered gloves is sufficient to mitigate the risks posed by these devices.

(Response 6) As described in Section IV of the proposed rule, FDA has determined that no change in labeling could correct the risk of illness or injury presented by the continued use of these devices. Powdered gloves have additional or increased risks to health compared to non-powdered gloves related to the spread of powder, and the fact that powder-transported contaminants such as NRL allergens can become aerosolized. Exposure to powder or latex allergens presents significant risks to health care workers and patients when inhaled or when exposed to internal tissue during oral, vaginal, gynecological, and rectal exams. Although labeling can raise awareness of these risks, we conclude that labeling cannot effectively mitigate these risks because it cannot prohibit the spread of glove powder or powder-transported contaminants. In addition, an important aspect of these devices is their ability to affect persons other than the individual who decides to wear or use them. For example, patients often do not know the type of gloves being worn by the health care professional treating them, but are still exposed to the potential dangers. Similarly, glove powder's ability to aerosolize and carry NRL proteins exposes individuals to harm via inhalation or surface contact. Thus, some of the risks posed by glove powder can impact persons completely unaware or unassociated with its employment and without the opportunity to consider the devices' labeling. Because of this inherent quality, adequate directions for use or warnings cannot be written that would provide reasonable assurance of the safe and effective use of these devices for all persons that might come in contact with them.

Due to the ability of powder to affect people who would not have an opportunity to read warning labels, and because potential warning labels would raise awareness of the risks, but would not eliminate the risks posed by glove powder, FDA has determined no label or warning can correct the risks posed by these devices.

(Comment 7) One comment opposes the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the solvent used to remove powder during the manufacture of non-powdered gloves may cause adverse reactions to the glove user.

(Response 7) FDA is not aware of any report in the literature that supports the assertion of widespread adverse reactions to solvent used in the manufacturing process. Non-powdered patient examination and surgeon's gloves require premarket notification (510(k)) submissions prior to marketing. During the review of these submissions, FDA evaluates the final finished glove, including manufacturing solvents that are present on the final glove. FDA recommends that manufacturers conduct and submit skin irritation and dermal sensitization studies in these submissions to evaluate potential issues with components, including manufacturing solvents (Ref. 1). Although individual hypersensitivity reactions to different materials may occur, FDA has been unable to find evidence in the literature of hypersensitivity to typical glove manufacturing materials other than glove powder or NRL. However, Palosuo, et al., reports that the use of hand sanitizers containing isopropyl alcohol prior to donning gloves could cause dermatitis reaction if the gloves are donned before the alcohol dries (Ref. 16). The occurrence of this reaction is unrelated to the manufacture of non-powdered gloves and unrelated to the use of non-powdered gloves as an alternative to powdered gloves. Given the lack of evidence of adverse reactions to solvents used in the manufacturing of non-powdered gloves, and the established evidence demonstrating the risks of powdered glove use, FDA continues to believe that powdered gloves and glove powder meet the banning standard.

(Comment 8) Several comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves due to the expectation that users will ultimately have to pay more for medical gloves once the ban is finalized, because the cost of non-powdered gloves is currently higher than the cost of powdered gloves.

(Response 8) We do not find any evidence to support the claims that

current prices of non-powdered gloves are significantly higher than powdered gloves. As we stated in the preliminary regulatory impact analysis (PRIA), extensive searches of glove distributor pricing indicate that non-powdered gloves have become as affordable as powdered gloves. Our searches also revealed that the market is saturated with alternatives to powdered gloves, resulting in downward pressure on the prices of non-powdered gloves. In addition, the share of powdered medical gloves sales has been declining since at least 2000 while total sales of all disposable medical gloves have increased (Ref. 17). We would not expect this trend to be occurring without regulatory action if users of disposable medical gloves faced significantly higher prices for switching to non-powdered gloves. We therefore do not find it necessary to update our analysis based on these comments.

(Comment 9) We received one comment that disagrees with our determination that the availability of examination and surgical gloves would not be reduced.

(Response 9) We do not find any evidence to support these claims. As we stated in the PRIA, research shows only 7 percent of total sales of examination and surgical gloves to medical workers were projected to be from powdered gloves in 2010 (Ref. 17). Global Industry Analysts (GIA) projected the share of powdered disposable medical gloves sales to decrease to 2 percent in 2015, while total sales of all disposable medical gloves continue to increase (Ref. 17). We would not expect this trend to be occurring without regulatory action if there were a reduction in the availability of disposable examination and surgical gloves. We therefore do not find it necessary to update our analysis based on these comments.

(Comment 10) Commenters suggest there would be a loss in consumer utility due to the preference some medical workers may have for powdered gloves due to comfort and ease of use.

(Response 10) We stated in the PRIA that the remaining 7 percent continuing to use these powdered gloves may experience utility loss from the removal of powdered gloves from the market (Ref. 17). The potential loss in consumer utility would be due to the perceived loss in comfort from powdered gloves users switching to non-powdered gloves. However, as the GIA report shows, there has been a downward trend in total sales of powdered gloves since at least the year 2000 while total sales of all disposable medical gloves has increased (Ref. 17). We would not expect this trend to be occurring without regulatory action if the loss in consumer utility to current medical workers were substantial. Korniewicz et al. reported no loss in consumer satisfaction in a sample of operating room staff switching to non-powdered surgical gloves (Ref. 4). We have not estimated this potential burden, but the evidence described here suggests that any burden would not be substantial. Further, even having considered that some degree of consumer comfort may be lost by banning powdered gloves, FDA continues to believe that this benefit is considerably outweighed by the numerous risks posed by powdered gloves.

(Comment 11) One comment opposes the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the risks identified for powdered gloves are due to contaminants, such as pesticides and herbicides, in the powder that would not be present if the powder were manufactured in the United States.

(Response 11) FDA disagrees with the assertion that contaminated powder is the source of the risks identified for powdered gloves. FDA's proposal to ban powdered gloves and glove powder is based on various studies on the risks of powdered gloves due to the properties of the powder itself. Powdered gloves have additional or increased risks to health compared to non-powdered gloves. For example, powder on NRL gloves can aerosolize latex allergens, resulting in sensitization to latex and allergic reactions. Latex sensitization and allergic reactions are unrelated to any potential presence of manufacturing contaminants, such as pesticides and herbicides. Additional risks of powdered gloves include severe airway inflammation, conjunctivitis, dyspnea, as well as granuloma and adhesion formation when exposed to internal tissue. FDA's assessment of the available literature and information indicates that these risks are attributable to the powder itself, as opposed to any potential presence of manufacturing contaminants, such as pesticides and herbicides.

In addition, the powder used on powdered gloves is required to comply with FDA's Quality System regulation, which includes requirements for quality and inspection for the final finished gloves that protect against the introduction of contaminated devices into commerce. Among other requirements, device manufacturers must establish and maintain procedures to prevent contamination of equipment or product by substances that could reasonably be expected to have an adverse effect on product quality (21 CFR 820.70(e)). FDA's Quality System regulation applies to gloves and glove powder sold in the United States, regardless of the manufacturing location.

D. Description of Comments on Scope of Ban and FDA Response

FDA received several comments requesting revision of the scope of the ban. The scope of the proposed ban includes powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove. The glove types include all powdered patient examination and surgeon's gloves, including NRL and synthetic latex gloves. In the following paragraphs, we discuss and respond to comments requesting revision of the scope of the ban. We are finalizing the ban without change to the scope, but clarifying that all powdered patient examination gloves and powder surgical gloves are banned, regardless of the material from which they are made.

(Comment 12) Several comments identify risks that result from the use of powdered and non-powdered NRL gloves. These comments request FDA to extend the ban to all NRL gloves, both powdered and non-powdered.

(Response 12) Unlike with powdered latex gloves, which have the ability to aerosolize glove powder and carry allergenic proteins, FDA believes the risk of allergic reaction to non-powdered NRL gloves, which affects the user and patients in direct contact with the glove, is adequately mitigated through already-required labeling that alerts users to this risk. NRL gloves must include a statement to alert users to the risk of allergic reactions caused by NRL (21 CFR 801.437). Further, several studies have indicated that the use of non-powdered NRL gloves reduces the risk of sensitization to allergenic NRL proteins and the number of allergic reactions experienced by those who are already sensitized (Refs. 18, 19, and 20). FDA believes that these study results, when considered alongside the risk mitigation that follows from FDA's required labeling for NRL products, demonstrates that non-powdered latex gloves can be safely used with appropriate caution for latex-sensitive patients and health care workers. Therefore, FDA has determined not to ban the use of all NRL gloves.

(Comment 13) Several comments raise the issue of life threatening latex allergy events that result from various uses of NRL gloves including food preparation and food service. Several of these comments assert that the Agency should broaden the scope of the ban to cover all

NRL gloves for all uses including food preparation and food service.

(Response 13) We have concluded that it is not appropriate to address a proposal to ban gloves used for food preparation because these gloves do not meet the definition of a device under section 201(h) of the FD&C Act and are thus not subject to section 516 of the FD&C Act (21 U.S.C. 360f), which provides the statutory authority to ban devices within FDA's authority to regulate such products.

(Comment 14) One comment asserts that the ban on powdered gloves should not apply to dental practice, because the risks are not applicable to dental practice.

(Response 14) FDA disagrees with the assertion that the risks of powdered gloves are not applicable to dental practice. Dentists and dental patients face the same risks as other medical practices in terms of the potential for powder exposure to open cavities or open wounds, and for powder, if used with NRL gloves, to carry protein allergens. Several studies documenting the risks of powdered gloves in dental practices have been conducted, including Saary, et al., which identified that changing to low-protein and non-powdered NRL gloves reduced NRL allergy in dental students (Ref. 18). In addition, Charous et al., reported in 2000 that a dental office was able to reduce airborne NRL antigen levels to undetectable levels with the exclusive use of non-powdered NRL gloves, permitting a highly sensitized staff member to continue to work there (Ref. 21). These studies, among others (Refs. 13 and 22), indicate that the risks of powdered medical gloves apply to dental practice. Therefore, FDA has determined that the scope of the ban on powdered medical gloves should continue to include powdered gloves used in dental practice.

E. Description of Other Specific Comments and FDA Response

Many comments made specific remarks requesting clarification or revision to the proposed rule. In the following paragraphs, we discuss and respond to such specific comments.

(Comment 15) A number of comments request extension of the effective date of the ban. The proposed rule included a proposed effective date of 30 days after publication of the final rule for all devices, including those already in commercial distribution. The comments suggest a range of effective dates of 90 days to 18 months after publication of the final rule and assert that a longer transition period is necessary to allow existing inventory to flow through the supply chain to providers and patients.

(Response 15) FDA is not extending the effective date of the ban for devices already in commercial distribution. We have concluded that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling. The continued marketing of these devices beyond the 30 day effective date would allow for the continued sale and purchase of devices that FDA has determined present an unreasonable and substantial risk to patients and health care workers. Therefore, FDA does not believe that it is in the best interest of the public health to extend the effective date for devices already in commercial distribution. In order to minimize the risk of continued exposure of health care workers and patients to these devices, the effective date for devices remains 30 days after the date of publication of this final rule.

(Comment 16) One comment requests that FDA not extend the effective date of the ban to allow companies to deplete their inventory of the devices.

(Response 16) As described in the response to comment 15, FDA agrees that it is in the best interest of the public health to not extend the effective date of the ban for devices already in commercial distribution. Therefore, the effective date of the ban for devices already in commercial distribution remains at 30 days after the date of publication of the final rule.

(Comment 17) A few comments request recommendations on the means of disposal or recycling of powdered gloves.

(Response 17) FDA recommends that unused inventories of powdered medical gloves remaining at domestic manufacturing and distribution locations be disposed of in accordance with standard industry practices. Unused supplies at hospitals, outpatient centers, clinics, medical and dental offices, other service delivery points (nursing homes, etc.), and in the possession of end users, will need to be disposed of according to established procedures of the local community's solid waste management system. Established procedures for these materials typically involve disposal in landfills or incineration. FDA has concluded that this final rule will not have a significant impact on the human environment. (See Section VII. Analysis of Environmental Impact.)

(Comment 18) One comment requests clarification on whether after the effective date of the ban the Agency will permit a manufacturer to export powdered medical gloves that are already physically located at distribution centers in the United States.

(Response 18) After the effective date of this final rule, manufacturers will not be allowed to import powdered medical gloves. However, while powdered medical gloves will be banned in the United States on the effective date of this final rule, manufacturers may export existing inventory of powdered gloves to a foreign country if the device complies with the laws of that country and has valid marketing authorization by the appropriate authority, as described in section 802 of the FD&C Act (21 U.S.C. 382)). If eligible for export under section 802 of the FD&C Act, a device intended for export will not be deemed adulterated or misbranded if it

(A) accords to the specifications of the foreign purchaser,

(B) is not in conflict with the laws of the country to which it is intended for export,

(C) is labeled on the outside of the shipping package that it is intended for export, and

(D) is not sold or offered for sale in domestic commerce.

V. Effective Date

This rule is effective January 18, 2017. The effective date of this rule applies to devices already in commercial distribution and those already sold to the ultimate user, as well as to devices that would be sold or distributed in the future. All powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's gloves must be removed from the market upon the effective date of this final rule. Section 501(g) of the FD&C Act (21 U.S.C. 351(g)) deems a device to be adulterated if it is a banned device.

VI. Economic Analysis of Impacts

A. Introduction

We have examined the impacts of the final rule under Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act (5 U.S.C. 601–612), and the Unfunded Mandates Reform Act of 1995 (Pub. L. 104–4). Executive Orders 12866 and 13563 direct us to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety,

and other advantages; distributive impacts; and equity). We have developed a comprehensive Economic Analysis of Impacts that assesses the impacts of the final rule. We believe that this final rule is not a significant regulatory action as defined by Executive Order 12866.

The Regulatory Flexibility Act requires us to analyze regulatory options that would minimize any significant impact of a rule on small entities. Because this rule imposes no new burdens, we certify that the final rule will not have a significant economic impact on a substantial number of small entities.

The Unfunded Mandates Reform Act of 1995 (section 202(a)) requires us to prepare a written statement, which includes an assessment of anticipated costs and benefits, before issuing ''any rule that includes any Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100,000,000 or more (adjusted annually for inflation) in any one year.'' The current threshold after adjustment for inflation is $146 million, using the most current (2015) Implicit Price Deflator for the Gross Domestic Product. This final rule would not result in an expenditure in any year that meets or exceeds this amount.

B. Summary of Costs and Benefits

The final rule prohibits marketing of powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating surgeon's gloves. The rule does not cover or include powdered radiographic gloves.

The final rule is expected to provide a positive net benefit (estimated benefits minus estimated costs) to society. Banning powdered glove products is not expected to impose any costs to society. Extensive searches of glove distributor pricing indicate that improvements to non-powdered gloves have made these products as affordable as powdered gloves. The ban is expected to reduce the adverse events associated with using powdered gloves. The Agency estimates maximum total annual net benefits to range between $26.8 million and $31.8 million. The present discounted value of the estimated benefits over 10 years ranges from $228.9 million to $270.8 million at a 3 percent discount rate and from $188.5 million to $223 million at a 7 percent discount rate.

FDA has examined the economic implications of the rule as required by the Regulatory Flexibility Act. If a rule will have a significant economic impact on a substantial number of small entities, the Regulatory Flexibility Act requires us to analyze regulatory options that would lessen the economic effect of the rule on small entities. This rule will not impose any new burdens on small entities, and thus will not impose a significant economic impact on a substantial number of small entities.

The full discussion of the economic impacts of the rule, which includes a list of changes made in the final regulatory impact analysis, in accordance with Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act, and the Unfunded Mandates Reform Act is available at *https://www.regulations.gov* under the docket number (FDA–2015–N–5017) for this rule and at *http://www.fda.gov/AboutFDA/ReportsManualsForms/Reports/EconomicAnalyses/default.htm*# (Ref. 23).

VII. Analysis of Environmental Impact

FDA has carefully considered the potential environmental effects of this final rule and of possible alternative actions. In doing so, the Agency focused on the environmental impacts of its action as a result of disposal of unused powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove that will need to be handled after the rule is finalized.

The environmental assessment (EA) considered each of the alternatives in terms of the need to provide maximum reasonable protection of human health without resulting in a significant impact on the environment. The EA considered environmental impacts related to landfill and incineration of solid waste at municipal solid waste (MSW) facilities nationwide. The selected action, if finalized, will result in an initial batch disposal of unused powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove from user facilities to MSW facilities nationwide, followed by a rapid decrease in the rate of disposal of these devices, as supplies are depleted. The selected action does not change the ultimate disposition of these devices but expedites their rate of disposal and ceases future production. Overall, given the limited number of powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove, currently in commercial distribution, the selected action is expected to have no significant impact on MSW and landfill facilities and the environment in affected communities.

The Agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The Agency's finding of no significant impact and the evidence supporting that finding, contained in an EA, may be seen in the Division of Dockets Management (see **ADDRESSES**) between 9 a.m. and 4 p.m., Monday through Friday (Ref. 24).

VIII. Paperwork Reduction Act of 1995

This final rule contains no collection of information. Therefore, FDA is not required to seek clearance by Office of Management and Budget under the Paperwork Reduction Act of 1995.

IX. Federalism

We have analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, we conclude that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

X. References

The following references are on display in the Division of Dockets Management (see **ADDRESSES**) and are available for viewing by interested persons between 9 a.m. and 4 p.m., Monday through Friday; they are also available electronically at *https://www.regulations.gov*. FDA has verified the Web site addresses, as of the date this document publishes in the **Federal Register,** but Web sites are subject to change over time.

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List of Subjects

21 CFR Parts 878 and 880

Medical devices.

21 CFR Part 895

Administrative practice and procedure, Labeling, Medical devices.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 878, 880, and 895 are amended as follows:

PART 878—GENERAL AND PLASTIC SURGERY DEVICES

■ 1. The authority citation for part 878 continues to read as follows:

Authority: 21 U.S.C. 351, 360, 360c, 360e, 360j, 360l, 371.

■ 2. Amend § 878.4460 by revising the section heading and paragraph (a) to read as follows:

§ 878.4460 Non-powdered surgeon's glove.

(a) *Identification.* A non-powdered surgeon's glove is a device intended to be worn on the hands of operating room personnel to protect a surgical wound from contamination. A non-powdered surgeon's glove does not incorporate powder for purposes other than manufacturing. The final finished glove includes only residual powder from manufacturing.

* * * * *

§ 878.4480 [Removed]

■ 3. Remove § 878.4480.

PART 880—GENERAL HOSPITAL AND PERSONAL USE DEVICES

■ 4. The authority citation for part 880 continues to read as follows:

Authority: 21 U.S.C. 351, 360, 360c, 360e, 360j, 371.

■ 5. Amend § 880.6250 by revising the section heading and paragraph (a) to read as follows:

§ 880.6250 Non-powdered patient examination glove.

(a) *Identification.* A non-powdered patient examination glove is a disposable device intended for medical purposes that is worn on the examiner's hand or finger to prevent contamination between patient and examiner. A non-powdered patient examination glove does not incorporate powder for purposes other than manufacturing. The final finished glove includes only residual powder from manufacturing.

* * * * *

PART 895—BANNED DEVICES

■ 6. The authority citation for part 895 continues to read as follows:

Authority: 21 U.S.C. 352, 360f, 360h, 360i, 371.

■ 7. Add § 895.102 to read as follows:

§ 895.102 Powdered surgeon's glove.

(a) *Identification.* A powdered surgeon's glove is a device intended to be worn on the hands of operating room personnel to protect a surgical wound from contamination. A powdered surgeon's glove incorporates powder for purposes other than manufacturing.

(b) [Reserved]

■ 8. Add § 895.103 to read as follows:

§ 895.103 Powdered patient examination glove.

(a) *Identification.* A powdered patient examination glove is a disposable device intended for medical purposes that is worn on the examiner's hand or finger to prevent contamination between patient and examiner. A powdered patient examination glove incorporates powder for purposes other than manufacturing.

(b) [Reserved]

■ 9. Add § 895.104 to read as follows:

§ 895.104 Absorbable powder for lubricating a surgeon's glove.

Absorbable powder for lubricating a surgeon's glove is a powder made from cornstarch that meets the specifications for absorbable powder in the United States Pharmacopeia (U.S.P.) and that is intended to be used to lubricate the surgeon's hand before putting on a surgeon's glove. The device is absorbable through biological degradation.

Dated: December 13, 2016.

Leslie Kux,

Associate Commissioner for Policy.

[FR Doc. 2016–30382 Filed 12–16–16; 8:45 am]

BILLING CODE 4164–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 880

[Docket No. FDA–2015–N–0701]

General Hospital and Personal Use Devices: Renaming of Pediatric Hospital Bed Classification and Designation of Special Controls for Pediatric Medical Crib; Classification of Medical Bassinet

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing a final rule to rename pediatric hospital beds as pediatric medical cribs and establish special controls for these devices. FDA is also establishing a separate classification regulation for medical bassinets, previously under the pediatric hospital bed classification regulation, as a class II (special controls) device. In addition, this rule continues to allow both devices to be exempt from premarket notification and use of the device in traditional health care settings and permits prescription use of pediatric medical cribs and bassinets outside of traditional health care settings.

DATES: This order is effective on January 18, 2017.

FOR FURTHER INFORMATION CONTACT: Michael J. Ryan, Center for Devices and Radiological Health, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 66, Rm. 1615, Silver Spring, MD 20993–0002, 301–796–6283.

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I. Executive Summary

A. Purpose and Coverage of the Final Rule

Pediatric medical cribs that meet the definition of a device in section 201(h) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 321(h)) (referred to as pediatric medical cribs or cribs intended for medical purposes) (product code FMS) are regulated by FDA and will have to comply with the special controls identified in this rule for pediatric medical cribs. Cribs that do not meet the device definition (referred to as cribs for non-medical purposes) must meet the Consumer Product Safety Commission's (CPSC's) regulations and guidelines.

In the **Federal Register** of December 28, 2010 (75 FR 81766), the CPSC issued a final rule prohibiting the use of the drop-side rail design for non-medical cribs in consumer households as of June 28, 2011. CPSC's rule established new standards for full-size and non-full-size cribs intended for non-medical purposes, which effectively prohibited the manufacture or sale of cribs intended for non-medical purposes with a drop-side rail design in households, child care facilities, family child care homes, and places of public accommodation. This rule did not affect pediatric medical cribs regulated by FDA, which typically contain a drop-side rail design that includes movable and latchable side and end rails. Although drop-side cribs intended for non-medical purposes are now prohibited, there is still a need for pediatric medical cribs with drop-side rails inside and outside of traditional health care settings. Pediatric medical cribs with drop-side rails are extremely helpful for patient care in hospital settings and even outside of traditional health care settings, such as day care centers caring for infants and children with disabilities, because they allow parents and care givers easy access to children to perform routine and emergency medical procedures, including, but not limited to, cardiopulmonary resuscitation (CPR), blood collection, intravenous (IV) insertion, respiratory care, and skin care. These drop-side rail cribs also make it easier for hospital staff to facilitate safe patient transport and reduce the chance of care giver injury.

Over the last 5 years, FDA has received over 500 adverse event reports, or Medical Device Reports (MDRs), associated with open pediatric medical cribs, through the Agency's Manufacturer and User Facility Device Experience (MAUDE) database. There were adverse event reports of serious injuries, including reports of entrapment, which were predominantly entrapments of extremities (legs or arms). The majority of MDRs for medical cribs were for malfunctions such as drop-side rails not latching or lowering, brakes not holding, wheels or casters breaking, and where applicable, scales not reading correct weights. As a result of the risks to health and need for continued use of pediatric medical cribs in traditional health care settings and non-traditional settings, FDA is revising the identification for § 880.5140 (21 CFR 880.5140) to include only pediatric

Exhibit 83

CLINICS 2007;62(5):627-34

REVIEW

INFLAMMATION AND CLINICAL REPERCUSSIONS OF PLEURODESIS INDUCED BY INTRAPLEURAL TALC ADMINISTRATION

Eduardo Henrique Genofre, Evaldo Marchi, Francisco S. Vargas

Genofre EH, Marchi E, Vargas FS. Inflammation and clinical repercussions of pleurodesis induced by intrapleural talc administration. Clinics. 2007; 62(5):627-34.

Although reports on pleurodesis date back to the beginning of the 20th century, the search for the ideal sclerosing agent is ongoing. Several agents have been studied and used, but talc continues to be the most popular. However, potentially harmful systemic side effects have been associated with talc pleurodesis. In this article we discuss the likely mechanisms of pleural inflammation and pleurodesis with emphasis on the systemic response due to the instillation of talc into the pleural space.

KEY-WORDS: Talc. Pleurodesis. Inflammation. Pleura. Pleural effusion.

Pleural effusion is a frequent manifestation, in part because of the increase in the incidence of cancer and, mainly, because of the survival of patients with malignant neoplasms.[1,2] Since the presence of pleural effusion is indicative of disseminated disease and limited survival, the main objective of treatment is to offer a better quality of life to the patients, relieving symptoms basically characterized by dyspnea and pain.[2] In addition, since systemic treatment frequently does not control the disease, the local approach to pleural effusion (in addition to drainage of the cavity for immediate improvement of manifestations) is the control of recurrence by sclerosis and symphysis of the pleural membranes, a process called pleurodesis.

The first reports on pleurodesis date back to the beginning of the 20th century,[3] and although more than 100 years have passed since then and different agents have been employed, the search for the ideal sclerosing agent continues.[4] Some antibiotics have demonstrated a sclerosing effect (tetracycline),[5] whereas no marked efficacy has been observed for others (macrolides and quinolones).[6] Similarly, antineoplastic agents (bleomycin),[7] immunostimulants (*Corynebacterium parvum* and OK-432),[8,9] chemical irritants (talc and silver nitrate),[10,11] and more recently, biological mediators of inflammation (transforming growth factor–beta (TGF-β) and interferon)[12,13] have been effective in the production of pleurodesis. The use of povidone-iodine,[14] autologous blood,[15] and polidocanol[16] has also been reported in some studies.

Among the different substances proposed for the induction of pleurodesis, talc is the agent most used in clinical practice. Talc, a magnesium silicate hydroxide [$Mg_3Si_4O_{10}(OH)_2$], has become popular because of its wide availability and high therapeutic success rate, which is close to 90%.[17] Talc is applied to the pleural space by injection in saline solution (slurry) or by dusting during pleuroscopy (poudrage).[18]

However, despite its widespread use, the instillation of talc is not devoid of side effects, including chest pain, fever, arrhythmias, and dyspnea.[18-20] Dyspnea is the most feared side effect because in more severe cases, this condition may result in acute respiratory distress syndrome, with an incidence reaching 9% and a potentially fatal outcome.[20,21]

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Systemic effects of talc

The mechanisms underlying the systemic effects of talc, especially respiratory distress syndrome, are still unknown. The possible and most likely causes include the migration of talc particles from the pleural cavity to the systemic circulation, changes provoked by the different talc components (including the presence of contaminating agents), establishment of an intracavity inflammatory process (source of inflammatory mediators), and surgical procedures performed (biopsies) that provide an access route that facilitates the systemic absorption of talc and inflammatory mediators.[22-25]

Migration of talc from the pleura to the systemic circulation

Considering the extensive surface of the human pleural cavity (≅ 1 to 2 m^2),[26] as well as the efficient drainage system of the cavity by pleural lymphatic vessels, the hypothesis that absorption of talc particles plays a role in the genesis of systemic effects should be taken into account.[23] Studies have shown heterogeneity in particle size among talc preparations used for pleurodesis.[27] Accordingly, one may speculate that smaller particles are more likely to be absorbed by the systemic circulation, causing the adverse effects described.[24,25,27]

In 1999, Werebe et al,[28] studying the dissemination of talc (particles measuring 5.7 to 70 μm) in rats, observed the presence of talc in bronchoalveolar lavage fluid and different organs (brain, liver, spleen, heart, and contralateral lung). Subsequently, in 2002, Ferrer et al[27] compared the induction of pleurodesis in rabbits using talc commonly employed in pleurodesis (particles of ≅ 8.3 μm) and talc with particles of a larger size (12 μm). The authors observed talc particles in the mediastinum pericardium and liver. Pleural inflammation and talc deposition were greater in the group receiving smaller particles. In the same year, Fraticelli et al,[29] using talc with larger particles (mean of 33 μm), identified the presence of a few talc particles in the brain, liver, and spleen, and a cross-contamination of these tissues during storage and processing of the samples was not excluded as a possible cause of the presence of talc in these tissues.

Our group also investigated the extrapleural dissemination of talc particles in rabbits, comparing small talc (mean particle size: 4.2 μm, with 50% of particles < 5 μm) and mixed talc (mean particle size: 25.4 μm, with 90% of particles > 10 μm) (Figure 1). We found talc deposition in the contralateral lung, liver, kidneys, and spleen, with a larger amount being observed in animals injected with talc comprised of small particles.[30,31]

Talc composition and contaminants

Another possible cause of systemic alterations is differences in talc composition, which varies in the amount of calcium, aluminum, and iron according to its origin. Similarly, mineral contaminants may produce local and systemic effects, with emphasis on the presence of magnesite, dolomite, caolinite, calcite, chlorite, serpentine, and quartz.[32]

Intracavity inflammatory process

Another possible cause of systemic side effects is the intense inflammatory process that is observed in the pleural space after talc application, producing cytokines, adhesion molecules, and other mediators of inflammation.[24,33]

The presence of local and systemic inflammatory alterations has been demonstrated in humans undergoing talc pleurodesis. In these patients, impaired DTPA clearance and changes in the alveolar-arterial oxygen gradient and serum

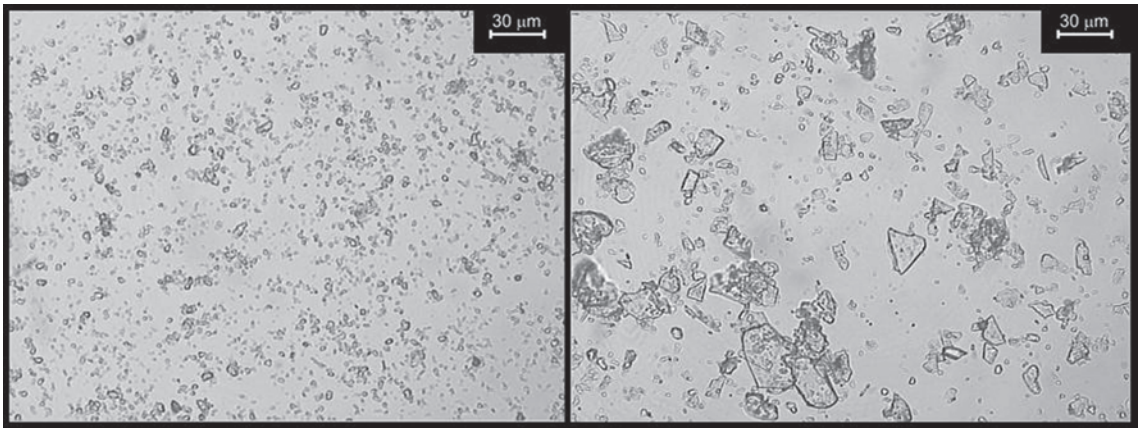

Figure 1 - Talc samples used. Left, talc containing small particles (< 5 μm). Right, mixed talc (90% of particles > 10 μm). Magnification: x400.

C-reactive protein levels were observed.[34,35] Additionally, chest computed tomography and positron emission tomography demonstrated alterations characteristic of acute pleural inflammation related to the loss of the integrity of the pleural barrier, consequently facilitating the absorption of talc particles and inflammatory mediators.[36]

Inflammatory response

The chain of events that leads to the occurrence of local and systemic effects is triggered by the inflammatory response to talc components or contaminants or even to inflammatory mediators produced as a result of intrapleural talc administration.[37] Thus, the understanding of this response is of great importance, taking into account cytological (particularly the acute response mediated by leukocytes and neutrophils), biochemical, and cytokine parameters. Analysis of the the response of leukocytes, and particularly that of neutrophils, is justified by the fact that a cellular response predominates in the pleural cavity, with lactate dehydrogenase being the main enzyme reflecting manifestations of an acute exudate.[37]

Main components

The inflammatory chain involves the production and release of modulatory substances that act on the producing cells themselves. In this respect, the key function of mesothelial cells was formerly believed to be the protection against aggression, with these cells being regarded as lining cells functioning as structural barriers. However, it is now known that mesothelial cells are not simple passive participants but are in fact responsible for the orchestration of the entire intrapleural inflammatory response, acting on the regulation of the production of diverse mediators such as interleukin 8 (IL-8), vascular endothelial growth factor (VEGF), and TGF-β.[37]

The predominant characteristics of IL-8, a cytokine involved in the acute inflammatory response, are neutrophil chemotaxis and marked resistance to proteolysis, with this cytokine also being associated with leukocyte activity during inflammatory processes in the pleural cavity.[38]

Vascular endothelial growth factor has 2 main properties, ie, the capacity to increase capillary permeability and a marked angiogenic and lymphogenic potential. It is present in inflammation areas and is found to be increased in exudative effusions, with this factor being implicated in the genesis and maintenance of pleural effusion.[37]

Tumor growth factor–beta presents profibrotic and immunomodulatory properties. Animal studies have demonstrated that TGF-β stimulates collagen synthesis and induces pleurodesis without causing acute inflammatory effects. Injection of TGF-β into the pleural space of rabbits increases VEGF levels in pleural fluid, and in vitro studies have shown that TGF-β stimulates the production of VEGF by mesothelial cells.[38]

Types of talc and inflammation

One of the main hypotheses raised to explain the systemic effects observed in pleurodesis is the migration of talc particles. Accordingly, smaller particles are believed to cause a more intense acute systemic inflammatory response than talc with particles of larger sizes. However, in fact, irrespective of particle size, talc injected into the pleural cavity produces an acute systemic response, with the presence of talc particles being rapidly detected in various organs (24 hours after intrapleural injection). Nevertheless, serum and pleural fluid inflammatory parameters indicate that small-particle talc (< 5 μm) produces a more prolonged and more intense systemic and pleural response.[28,39] Therefore, talc administration leads to an increase in the serum levels of IL-8, VEGF, and TGF-β and in pleural levels of IL-8 and VEGF, irrespective of particle size, with these levels being more elevated when small-particle talc is injected.[30] Nasreen et al[40] demonstrated that mesothelial cells stimulated by talc produce adhesion molecules and cytokines, emphasizing the importance of talc for the mechanism of pleural inflammation in pleurodesis.

In clinical studies, Maskell et al[35] demonstrated that talc of varying particle size (50% of particles < 10 μm) exerts more pronounced effects on the lung clearance of DTPA and serum IL-8 and C-reactive protein levels than tetracycline, in addition to causing more lung and systemic alterations. Another study of the same group compared pleurodesis obtained with mixed talc and graded talc in which 50% of the particles were smaller than 20 μm, with most particles smaller than 10 μm being removed for homogenization of the sample. Measuring the alveolar-arterial oxygen gradient and C-reactive protein levels, the authors demonstrated more systemic and lung inflammation, accompanied by worsened gas exchange, in pleurodesis induced with small-particle talc. The final outcome of pleurodesis was the same after 3 months (85% success in both groups).[34] Recently, Froudarakis et al[41] evaluated the role of talc in the systemic inflammatory response comparing diagnostic thoracoscopy and thorascopic talc poudrage. Talc was found to actively participate in the systemic reactional process, causing an increase in leukocyte count and in the percentage of neutrophils, associated with fever. In addition, C-reactive protein levels were significantly higher in the group submitted to talc poudrage.

The above mentioned facts are important, since no standardization exists for talc production, and there is a wide variation in the composition and particle size among the various talc preparations used worldwide. This fact has been demonstrated by Ferrer et al,[27] who attributed side effects to the smaller size of the particles in the talc used in different services. A similar efficacy of pleurodesis was reported by the same group when comparing 200 mg/kg of talc used in clinical practice (particle diameter: 8.36 ± 0.20 μm) and talc with particles of larger size (diameter: 12 ± 0.25 μm), but pleural inflammation was greater when the injected talc contained smaller particles.[27,32] Montes et al,[25] comparing 2 different talc doses (50 or 200 mg/kg), noted a more intense systemic response with the higher dose, including an increase in the number of lymphocytes, monocytes, neutrophils, and platelets.

The more intense pleural inflammation and the more prolonged systemic response observed after the administration of small talc (50% of particles < 5 μm), leads to different speculations regarding the possible mechanisms of inflammation. We believe that the greater pleural inflammatory response observed after the administration of talc containing small particles is a consequence of the larger number of particles per area in the cavity. Furthermore, smaller particles probably are more easily engulfed by inflammatory cells present in the pleural space, in the absence of intense cellular necrosis, a fact that might explain the observation of a larger number of intact neutrophils, the lower lactate dehydrogenase levels, and the more marked release of inflammatory mediators (cytokines). On the other hand, mixed talc (90% of particles > 10 μm) presents a rough spike-like shape and may cause more intense damage to mesothelial cells by a direct mechanical action, resulting in greater cellular necrosis accompanied by increased lactate dehydrogenase levels.[30]

Systemic inflammatory response

Regardless of the type of talc used, the systemic response observed in pleurodesis is believed to be the result of the passage of inflammatory mediators or talc particles from the pleura to the blood circulation. This passage may occur through the lymphatic route[23, 26, 27] (drainage into the venous system and deposition in the lungs) or by direct passage into the bloodstream due to the loss of integrity of the alveolar-capillary barrier secondary to the intense inflammation in the pleural cavity.[10]

The first mechanism, ie, spilling of inflammatory mediators, is supported by the view that in the pleural cavity, IL-8 levels increase early and decline over time, whereas the opposite is observed in blood.[10] In parallel, VEGF levels tend to increase progressively in both pleural fluid and blood, reflecting an increase in capillary permeability, which contributes to both the production of pleural fluid and the passage of smaller molecules from the cavity to the blood circulation. We observed that only TGF-β levels after instillation of small talc (50% of particles < 10 μm) and VEGF levels after administration of mixed talc (90% of particles > 10 μm) presented a strong pleura-serum correlation (Figure 2), suggesting a possible communication between the 2 compartments.[30,41-43] Despite this observation, these data alone do not permit the inference that this is the only mechanism involved in the systemic response observed in talc pleurodesis.

Comparison of these parameters shows a positive correlation between VEGF and TGF-β levels in pleural fluid, demonstrating a possible proinflammatory effect of TGF-β. This possibility has been suggested in a previous study in which TGF-β was found to stimulate the production of VEGF by mesothelial cells, a finding explaining in part the increased vascular permeability observed in inflammation

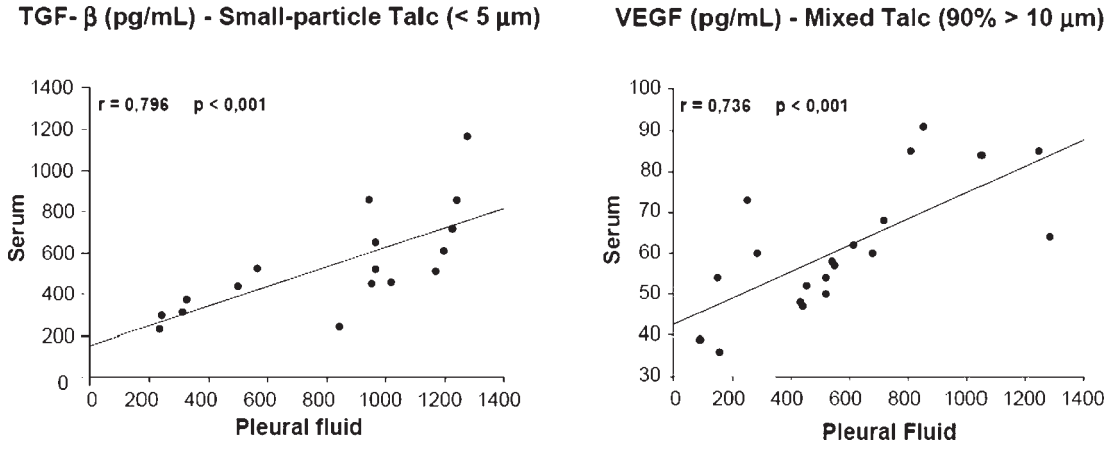

Figure 2 - Comparison of tumor growth factor–beta (TGF-β) and vascular endothelial growth factor (VEGF) levels in pleural fluid and blood of rabbits submitted to intrapleural injection of small-particle talc and mixed talc, respectively.

after pleurodesis.[43] However, we observed higher pleural VEGF levels after the administration of small talc (< 5 μm) compared with mixed talc (90% of particles > 10 μm), whereas TGF-β levels were higher in animals injected with mixed talc, suggesting a possible overlapping of distinct inflammatory mechanisms.[30,44]

In addition to inflammatory mediators, an acute serum increase of leukocytes and neutrophils is also observed in both talc groups, coinciding with the acute pleural inflammatory response. However, no conclusion can be drawn about whether the systemic cellular response is the result of the flow of cells from the pleural cavity to the blood circulation or whether it is a direct response to the presence of talc particles in the organs.[30,31] Therefore, future studies correlating the cellular response with the number of particles deposited in tissues will contribute to clarifying this question.

Another mechanism that might explain the systemic inflammatory response is the direct passage of talc particles from the pleural space into the blood circulation. The presence of talc particles in intrathoracic and extrathoracic tissues has been the subject of many studies. Ferrer et al[27,32] suggested that small particles are more easily absorbed by lymphatic stomata (which, in humans, measure about 6.2 μm in diameter[26]) and are carried by lymphatic vessels, triggering an inflammatory response that provokes acute lung injury in the form of adult respiratory distress syndrome. This assumption is justified by the fact that all series in which cases of respiratory insufficiency were reported were from North America (a country presenting the smallest particles among those studied). Curiously, no case of adult respiratory distress syndrome was reported in 2 large series (one European and another Israeli series).[24,32]

Our studies agree with the previously described findings and emphasize the observation that the particles present in the organs studied are all smaller than 5 μm, suggesting that the systemic effects observed can be explained by the presence of small particles. The same mechanism may explain the systemic effects observed after the administration of mixed talc, since about 10% of the particles in this type of talc are smaller than 10 μm. However, if we assume that the probable mechanism of talc particle migration from the pleural cavity to the systemic circulation involves lymphatic absorption, it would be difficult to explain the presence of talc particles in the liver, spleen, and kidney (Figure 3), because these particles enter the systemic venous circulation and return to the lung where they are retained. Therefore, we believe that lymphatic absorption is not the only mechanism involved, and we speculate that the intense inflammation caused by talc promotes the loss of integrity of the capillary barrier, permitting the free flow between the 2 compartments.

These considerations permit us to infer that the mechanism underlying the systemic inflammatory response ob-

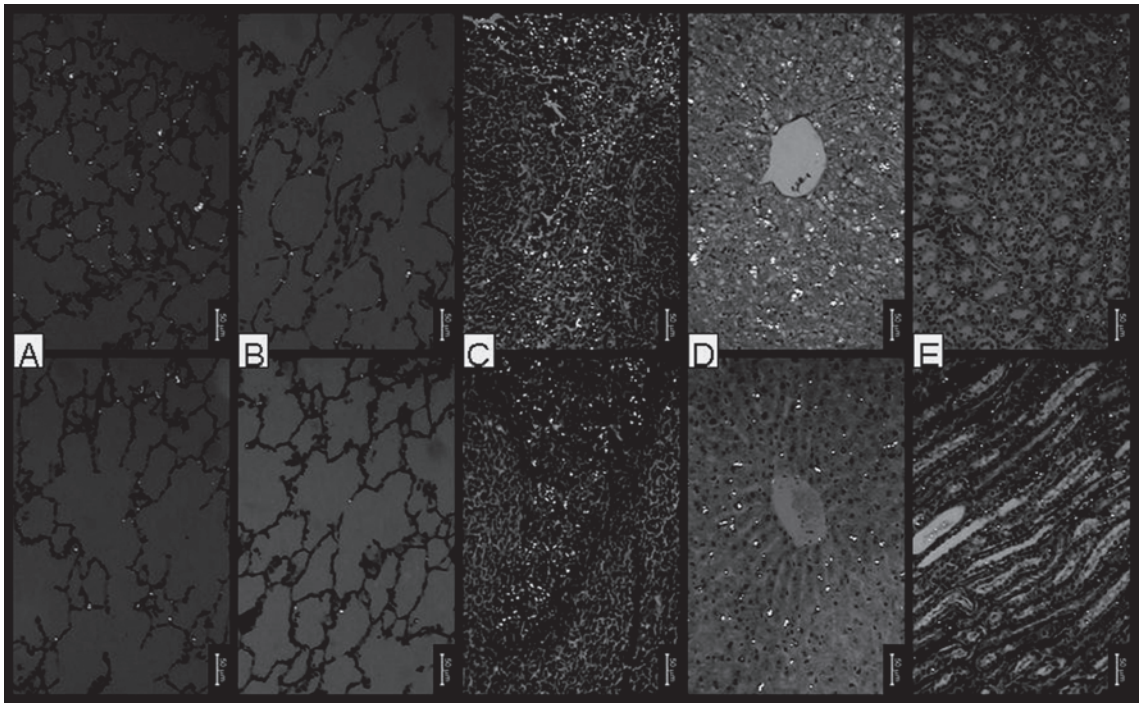

Figure 3 - Photomicrographs of the left (A) and right lung (B), spleen (C), liver (D), and kidney (E) of rabbits submitted to th intrapleural instillation of small-particle talc (< 5 μm) (upper panel) and mixed talc (90% of particles > 10 μm) (lower panel) obtained after 24 hours (hematoxylin and eosin, polarized light, magnification: x200). Note the scattered birefringent talc particles observed in both images.

served in talc pleurodesis remains to be established. However, it seems reasonable to assume that particle size influences the inflammatory response and, possibly, the side effects observed in clinical practice, since talc migration to other organs has been demonstrated.

We conclude that talc-containing particles of small size cause more marked pleural inflammation and a greater systemic inflammatory response, accompanied by extrapleural talc deposition in various organs. The mechanisms underlying these phenomena are still not completely understood, and further studies on this subject are necessary, especially those investigating the mechanisms of talc particle migration.

AUTHORS' NOTE

The talc used by the Discipline of Pneumology, University of São Paulo Medical School (HC-FMUSP), comprises a wide variation in particle size, with about 10% consisting of particles smaller than 10 μm. Considering that about 5 g of talc is instilled during pleurodesis induction, about 500 mg of this agent comprises particles smaller than 10 μm. Thus, for a pleural surface of about 2000 cm^2, 500 mg of small-particle talc corresponds to 4 mg/cm^2 (4000 $\mu g/cm^2$), a dose considered to be extremely high and potentially toxic to the mesothelium. We demonstrated in cell culture experiments that doses higher than 48 $\mu g/cm^2$ provoke mesothelial cell necrosis.[44,45]

RESUMO

Genofre EH, Marchi E, Vargas FS. Repercussões clínicas e inflamatórias de pleurodese induzida pela administração intrapleural de talco. Clinics. 2007; 62(5):627-34.

Apesar dos relatos sobre pleurodese remontarem ao início do século XX, ainda hoje se busca o agente esclerosante ideal. Diversos agentes foram estudados e utilizados, mas o talco é considerado o mais popular. No entanto, efeitos sistêmicos potencialmente tóxicos tem sido associados à pleurodese pelo talco. Neste artigo discutimos os prováveis mecanismos de inflamação pleural e pleurodese, com ênfase na resposta sistêmica produzida pela instilação intrapleural de talco

UNITERMOS: Talco. Pleurodese. Inflamação. Pleura. Efusão Pleural.

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Exhibit 84

Correspondence

ERRATUM: SMOOTH REFERENCE EQUATIONS FOR SLOW VITAL CAPACITY AND FLOW–VOLUME CURVE INDEXES

From the Authors:

Upon notification from a reader of our article (1), the authors have found three incorrect coefficients in Table 3, on page 901 entitled "Coefficients associated to the independent variables estimated for different regressions of eight lung function indices separately for the two sexes." The incorrect coefficients are those in the "Height-squared" row for "VC", "FEV_1", and "FVC" in males.

The published coefficients cause erroneous predicted values, as shown in the following example for a male 40 years of age whose height and weight are 180 cm and 75 kg, respectively:

	Published Incorrect Coefficient	Correct Coefficient
VC predicted, L	−8.32	5.60
FEV_1 predicted, L	−10.10	4.24
FVC predicted, L	−7.01	5.53

We had not previously discovered the errors because we kept applying the reference equations by using the values directly derived from the output of the datafile, instead of those published.

We apologize for the mistake, which was not due to the printing procedures of the *American Journal of Respiratory and Critical Care Medicine*, but to an error in the transcription of the coefficients from the output of the datafile to the original submitted manuscript.

We include below the relevant part of Table 3, after correction. We think that it may be more useful for the readers rather than publishing only the correct coefficients by themselves.

Francesco Pistelli

Institute of Clinical Physiology
Pisa, Italy

1. Pistelli F, Bottai M, Viegi G, DiPede F, Carrozzi L, Baldacci S, Pedreschi M, Giuntini C. Smooth reference equations for slow vital capacity and flow–volume curve indexes. *Am J Respir Crit Care Med* 2000;161:899–905.

TABLE 3. COEFFICIENTS ASSOCIATED TO THE INDEPENDENT VARIABLES ESTIMATED FOR DIFFERENT REGRESSIONS OF EIGHT LUNG FUNCTION INDICES SEPARATELY FOR THE TWO SEXES

	VC	FEV_1	FVC
Males			
Constant	12.11066	6.760398	11.70785
BMI	0.2394851	0.1879899	0.2819616
BMI-squared	−0.0043891	−0.0036597	−0.0054501
Height	−0.218829	−0.1368342	−0.2186871
Height-squared	0.000821	0.000548	0.0008324
Age	0.1927431	0.1459458	0.1659875
$Spline_1$ (Age)	0.0011271	0.0010309	0.0011508
$Spline_2$ (Age)	−0.0007911	−0.0007106	−0.0008498
(break-points)	(20, 25)	(19, 24)	(20, 24)
Normal 95th percentile, %	83	82	82
95% confidence interval	81–85	80–84	80–84

INCREASED MATRIX METALLOPROTEINASE (MMP)-9 IN THE AIRWAY AFTER ALLERGEN CHALLENGE

To the Editor:

In regard to the article by Kelly and colleagues (1) showing increased gelatinase B or matrix metalloproteinase (MMP)-9 levels and lack of its activation in airway after allergen challenge, we wish to point out the crucial role of timing in the collection of bronchoalveolar lavage fluid (BALF) when assessing the degree of MMP-9 activation following allergen challenge studies. Increased levels and degree of activation of neutrophil-derived MMPs (MMP-8 and MMP-9) in particular in BALF have been shown to be associated with human and equine inflammatory lung diseases (2–4), and pathologically elevated levels of MMP activation may reflect the active phase of lung inflammation as well as the severity of the ongoing inflammatory response.

Kelly and colleagues (1) demonstrated that increased levels of MMP-9, eventually originating from degranulating activated neutrophils, was present in allergen-challenged airways mainly in latent form, as determined from BALF collected 5 min and 48 h after challenge. In our recent study, applying different and more frequent timing of BALF sample collection (0 h, 5 h, 24 h, and 48 h) we have demonstrated a significantly elevated degree of activation of MMP-9 when horses with heaves (organic dust-induced asthma) in disease remission were challenged with inhaled organic dusts and inhaled endotoxin. The significant activation of MMP-9 was evident especially in BALF collected at 5 h after challenge, and was accompanied by elevation of proMMP-9, whereas elevation of proMMP-9, but not activation of MMP-9, was still evident 48 h after challenge. Additionally we have demonstrated pathologically elevated and activated levels of both MMP-9 in BALF from steroid-naive human asthmatic patients (median [range]; asthmatics: n = 10, pro-MMP-9 3.10 [1.04–9.18], active MMP-9 0.14 [0–3.73]; healthy controls: n = 10, pro-MMP-9 1.45 [0–2.52], active MMP-9 0 [0–0]) and MMP-8 in BALF collected from human bronchiectatic patients (2). In agreement with Kelly and coworkers (1) the functional significance of MMP-9 in BALF remains to be determined. Nonetheless, appropriate timing of BALF sampling in relation to allergen challenging evidences MMP-9 activation and permits investigation and identification of the potential pro-MMP-9 activators such as serine proteinases and/or reactive oxygen metabolites (5, 6). Furthermore, MMP-9 activation and its activators may prove to be useful tools to diagnose the active phase of inflammatory lung diseases.

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From the Authors:

We thank Dr. Maisi and colleagues for their comments concerning the kinetics of the presence of activated MMP-9 in the airway following broncho-

provocation. In our recently published study (1), we demonstrated increased bronchoalveolar lavage (BAL) fluid levels of MMP-9 48 h after airway allergen challenge. Our intent was not to determine the activation status of MMP-9; however, zymographic analysis suggested a predominance of the latent form. Dr. Maisi has kindly provided us with preprints of his submitted manuscripts (2–4). In these studies, he and his colleagues have shown increased BAL fluid levels of MMP-9, but not MMP-2 or MMP-14 in horses with "chronic obstructive pulmonary disease" compared with normal healthy animals (2). In another study, horses with "COPD" were given an inhalation challenge with moldy straw/hay dust or lipopolysaccharide (LPS) and BAL was performed at 5 h, 24 h, 4 d, 7 d, and 14 d after challenge (3). At 5 h after challenge, dust or LPS-challenged horses showed increased BAL fluid levels of the activated form of MMP-9. They also conducted studies in patients with bronchiectasis. Both pro and latent MMP-8 were detectable in BAL fluid (Western blot analysis) and immunoreactive MMP-8 (immunohistochemistry) in bronchial biopsies from these patients. Studies of asthmatics were not included in this report. It should be emphasized that the studies by Maisi differ from ours in a number of key aspects. First, our study was conducted in atopic humans challenged with allergen, their study in bronchiectasis patients or in horses challenged with hay dust or LPS. Second, our protocol was designed to induce airway eosinophilia, their protocol was designed to induce airway neutrophilia. Finally, because of safety concerns for our human subjects, BAL was only performed at 5 min and 48 h (time of peak airway eosinophilia) after challenge, while BAL was performed at 5 time points, including 5 h post challenge in their study of horses.

Our study clearly demonstrated that MMP-9 is increased 48 h after allergen challenge of atopic individuals; however, we agree with Dr. Maisi, that we might have seen more active enzyme if BAL were performed 4–6 h after challenge, as this time point typically coincides with peak airway neutrophilia (5, 6). It is not clear whether the persistence of the latent form at 48 h is merely a marker of an earlier influx of airway neutrophils, or if it can be subsequently activated to contribute to tissue injury and repair.

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TALC SHOULD NOT BE USED FOR PLEURODESIS IN PATIENTS WITH NONMALIGNANT PLEURAL EFFUSIONS

To the Editor:

In the debate regarding the use of talc in pleurodesis, respiratory failure after intrapleural injection was cited as that complication potentially limiting employment of this agent (1, 2). We agree with this appraisal in the treatment of patients with malignant recurrent effusions. However, there should continue to be concern regarding the use of talc for pleurodesis in individuals with nonmalignant pleural effusions and spontaneous pneumothorax. This dilemma results from a possible increased risk of malignant mesothelioma in those patients treated with talc. Consequently, an alternative agent should be employed in any individual without malignancy requiring pleurodesis.

Talc is not a uniform substance, and varies significantly in size and chemical composition, with the latter depending on geologic origin. This sheet silicate can be contaminated by asbestos. An association between carcinogenesis and exposure to asbestos included in talc appears credible. Certainly, noncarcinogenic effects of asbestos (pleural plaque formation) have been reported in patients instilled with talc for pleurodesis. The paucity of evidence of malignant mesothelioma occurring after the use of talc for pleurodesis may reflect either an inadequate latency period or an insufficient number in the investigations conducted. Assuming a risk of the same magnitude as that seen in the cohort of asbestos-exposed insulation workers (3), less than one case of mesothelioma would have been expected in the two investigations of patients exposed to talc used in pleurodesis (4, 5). However, case reports of malignant mesothelioma after occupational exposure to talc suggest a potential association (6). Furthermore, epidemiologic studies demonstrate an excess mortality from lung and pleural carcinomas in talc miners and millers, while animal studies confirm an induction of mesothelioma after intrapleural injection of talc.

The assertion that contemporary purified preparations of talc do not contain asbestos, therefore eliminating a risk of mesothelioma, should be closely examined prior to its acceptance for clinical application. The methodology used to confirm the lack of asbestiform minerals in a finished product (i.e., X-ray diffraction, optical microscopy, and electron microscopy techniques) and its sensitivity must be provided. Even if the product is "asbestos-free," the mechanism of cancer induction by asbestos (i.e., metal-catalyzed radical generation) is similarly pertinent to talc and the occurrence of fibrous forms of the sheet silicate itself (Figures E1 and E2 in the online data supplement to this letter) raises issues about clearance and long-term safety. Simply stating that the talc is "asbestos-free" should not release us from a responsibility to the patient, especially when safe alternatives are available.

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Chapel Hill, North Carolina

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Durham, North Carolina

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From the Authors:

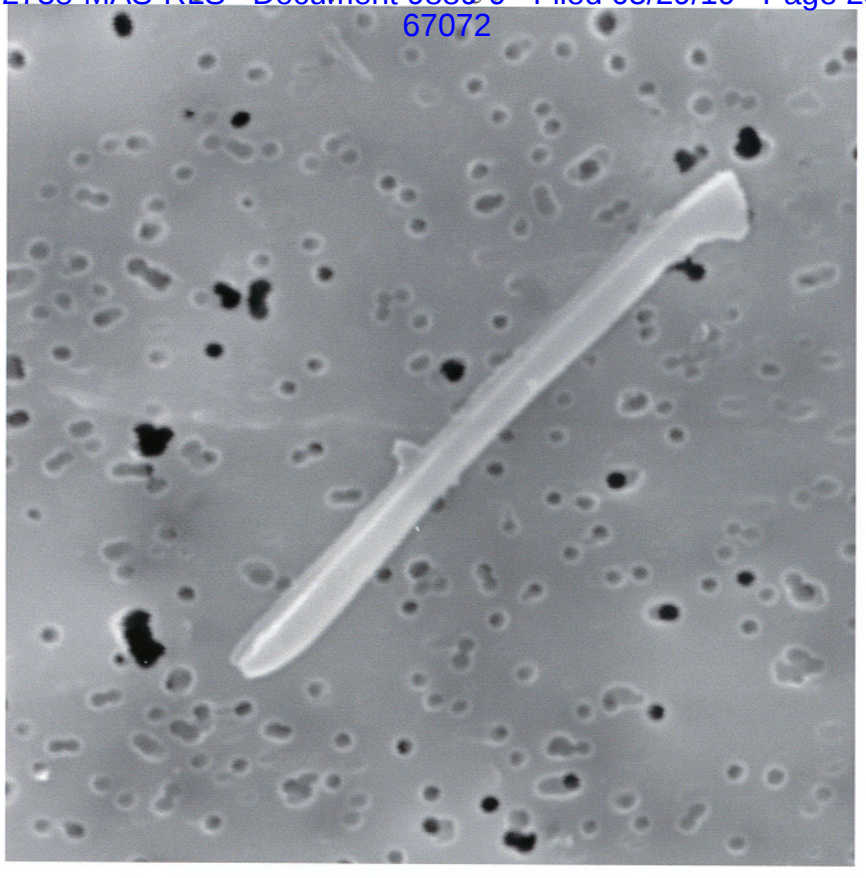
I appreciate the comments of Drs. Ghio and Roggli concerning our article. I agree that talc should not be used to produce pleurodesis in patients with nonmalignant diseases such as spontaneous pneumothorax or recurrent nonmalignant pleural effusion. If talc should not be used to produce pleurodesis in patients with malignant disease because it might produce acute respiratory failure, it should not be used for pleurodesis in other situations for the same reasons.

Drs. Ghio and Roggli maintain that another reason talc should not be used in patients with nonmalignant disease is the possible increased risk of mesothelioma after the administration of talc intrapleurally. Talc can be contaminated with asbestos, which is known to be associated with the development of mesothelioma. Although previous studies have found no increased incidence of mesothelioma in patients who received talc intrapleurally, the authors rightly point out that the number of patients included in the studies was small. I believe that the risk of mesothelioma from talc pleurodesis is very small since, to my knowledge, there is not even a case report of such an occurrence. Nevertheless, the fact that the possibility exists provides another reason to not use talc for pleurodesis in nonmalignant conditions.

Richard W. Light
Vanderbilt University
Nashville, Tennessee

Dr. Sahn was given an opportunity to respond, but declined.

FigE1 Secondary Electron Image, X 5500



FigE2. Energy Dispersive X-ray Spectrum

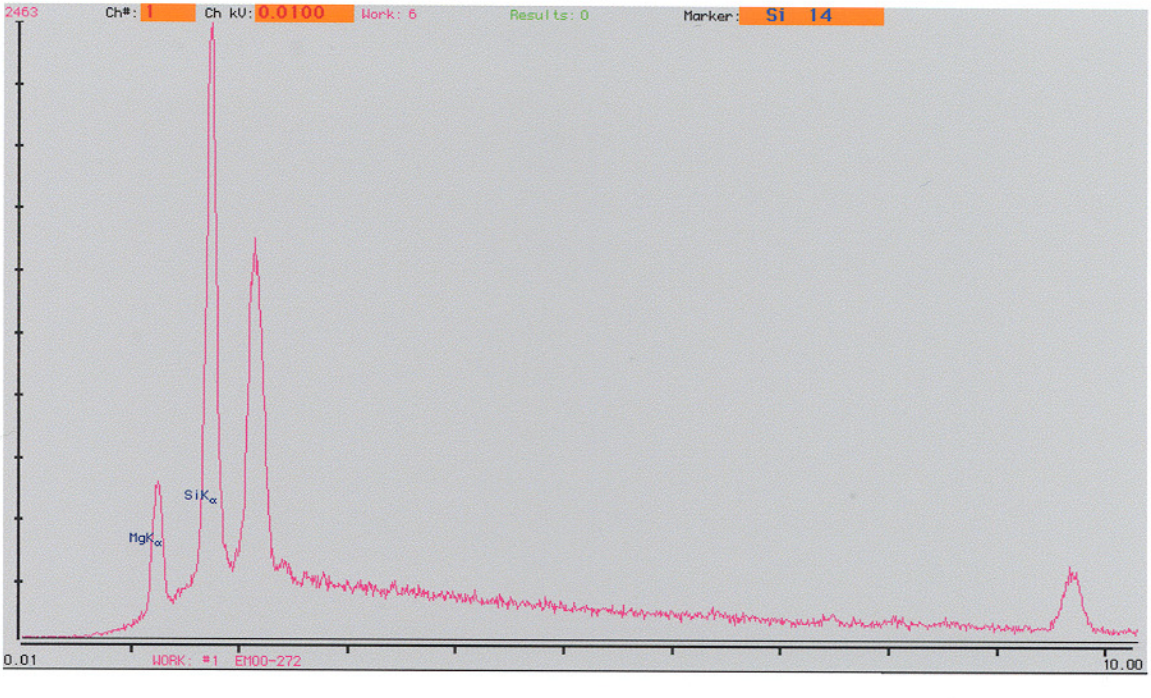


Figure E1. Talc structure isolated from pleura of patient undergoing talc pleurodesis. The aspect (length to width) ration of this structure exceeds 10.

Figure E2. The composition of the fiber y energy dispersive spectrometry indicates that it is a magnesium silicate, consistent with talc. The two unlabeled peaks are gold.

Exhibit 85

Foreign Body Granulomas in Normal Ovaries

S. A. M. MOSTAFA, MD, C. B. BARGERON, PhD, R. W. FLOWER, BA, N. B. ROSENSHEIN, MD, T. H. PARMLEY, MD, AND J. D. WOODRUFF, MD

In 100 consecutive cases in which grossly normal ovaries were removed at the time of pelvic surgery, 9% were found to contain crystalline foreign particles. An additional 9% contained cortical granulomas. In four of six cases, computer-assisted x-ray analysis of the crystalline foreign particles was successful and revealed magnesium and silicon. ***(Obstet Gynecol 66:701, 1985)***

To make plausible the suggestion that inorganic particulate matter plays a role in the development of proliferative disorders in the female pelvis,[1–5] it is necessary to demonstrate that such matter is capable of producing proliferations under some circumstances. It is also necessary to demonstrate that particulate matter is actually present in the female pelvis with sufficient frequency to account for the amount of observed disease. The present study was designed to address this second question; it does not address the first. It also seeks to determine the elemental nature of the particles observed.

Materials and Methods

In 100 consecutive cases in which grossly normal ovaries were removed at the time of pelvic surgery for other indications, the entire gonad(s) was submitted for histologic examination. A total of 175 normal ovaries were examined. Two to five paraffin blocks were made from each excised gonad, and an average of three sections per ovary were studied. Findings in these 175 ovaries were divided into four groups: cases in which there were no histologic abnormalities, group 1; cases in which there were laminated calcifications, classically referred to as "psammoma bodies," group 2 (Figure 1); cases in which there were foci of reticular stroma with or without inflammation that have been classically referred to as "cortical granulomas," but have been described as endometriosis by others,[6] group 3 (Figure 2); and cases in which foci similar to those in group 3 appeared and which additionally contained foreign body type giant cells and associated crystalline foreign body, group 4 (Figure 3). If two ovaries were removed from one patient, they were classified together as a single case.

Figure 1. Two laminated focal calcifications occupy papillary fronds in this proliferating pelvic neoplasm.

Six examples of crystalline foreign bodies were then processed for examination by scanning electron mi-

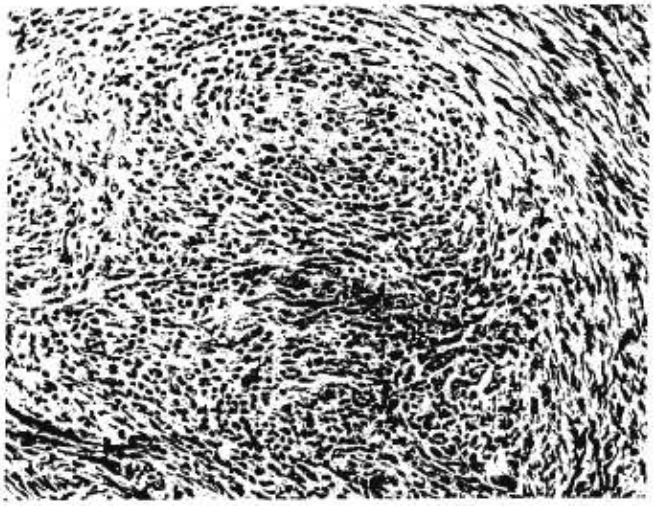


Figure 2. This focus of inflammation was present in the ovarian cortex. Although granulomatous in nature, no giant cells or particulate foreign matter is observed.

From the Department of Gynecology and Obstetrics, The Johns Hopkins Hospital, Baltimore, Maryland.

0029-7844/85/$3.30

Figure 3. This focus of inflammation in the ovarian cortex contains giant cells. The clefts within the giant cells were filled with refractile crystalline material (*arrows*).

croscopy (ETEC microscope). This consisted of making a number of visible light micrographs in order to record the location of foreign bodies in the specimen and then removing the slide cover slips. The exposed specimens were then mounted on an electron microscope slide and carbon coated. Using appropriate computer-assisted microscopic x-ray analysis, the elemental composition of the crystalline foreign bodies were determined in four cases. In the two other cases, the foreign body was lost when the cover slip was removed.

Results

One hundred seventy-five grossly normal ovaries were removed at the time of pelvic surgery. The surgery was performed for the indications listed in Table 1. Seventy-two cases were classified in group 1 (Table 2).

Computer-assisted x-ray analysis of the crystalline foreign bodies was successful in four of six cases and demonstrated that the particles were composed largely of magnesium and silicon. The mean ages of each group and the percentage with a history of laparotomy also are listed in Table 2.

Table 1. Pelvic Surgery for Various Gynecologic Disorders

Diagnosis	No. of cases
Myomata uteri	42
Endometrial carcinoma	18
Cervical carcinoma	10
Endometrial & cervical carcinoma	1
Mixed mesodermal tumor of cervix	1
Uterine leiomyosarcoma	1
Adenomyosis	3
Parovarian cyst	2
Unilateral ovarian neoplasm	5
Endometrial polyp hyperplasia	1
Salpingitis	4
Pelvic endometriosis	3
Chronic pelvic pain	2
Pelvic inflammatory disease	1
Dysfunctional uterine bleeding	6
Total	100

Table 2. Findings in 100 Consecutive Cases in Which a Grossly Normal Gonad(s) Was Removed

Group	No. of cases	Mean age	% Previous laparotomy
1	72	44	36
2	10	50	30
3	9	52	22
4	9	62	44

Discussion

The most common compounds containing magnesium silicates in industrial North America are talc and asbestos. As reported,[1–3,5] it is not a new observation that talc may be found in the pelvis, nor are talc granulomas in and of themselves new observations. However, the fact that 9% of the women operated on in the Johns Hopkins Hospital for pelvic disease appeared to have magnesium silicate granulomas in their normal ovaries, and that an additional 9% contained histologic entities that were very similar, represents a higher incidence than the authors had suspected. The exact figure is probably not relevant as it, undoubtedly, varies from population to population, depending on the exposure sustained by that given population. Nevertheless, in at least one geographic area, the incidence of foreign body contamination in the pelvis is sufficiently high to account for the incidence in that geographic locale of proliferative disorders seen at that anatomic site.

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Exhibit 86

Reproductive Biology and Endocrinology

BioMed Central

Review

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Animal models of ovarian cancer

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Abstract

Ovarian cancer is the most lethal of all of the gynecological cancers and can arise from any cell type of the ovary, including germ cells, granulosa or stromal cells. However, the majority of ovarian cancers arise from the surface epithelium, a single layer of cells that covers the surface of the ovary. The lack of a reliable and specific method for the early detection of epithelial ovarian cancer results in diagnosis occurring most commonly at late clinical stages, when treatment is less effective. In part, the deficiency in diagnostic tools is due to the lack of markers for the detection of preneoplastic or early neoplastic changes in the epithelial cells, which reflects our rather poor understanding of this process. Animal models which accurately represent the cellular and molecular changes associated with the initiation and progression of human ovarian cancer have significant potential to facilitate the development of better methods for the early detection and treatment of ovarian cancer. This review describes some of the experimental animal models of ovarian tumorigenesis that have been reported, including those involving specific reproductive factors and environmental toxins. Consideration has also been given to the recent progress in modeling ovarian cancer using genetically engineered mice.

Introduction

Despite improved knowledge of the etiology of ovarian cancer, aggressive cytoreductive surgery, and modern combination chemotherapy, there has been little change in the mortality statistics over the last 30 years, and approximately 60% of the women who develop ovarian cancer will die from their disease. Lack of an adequate screening test for early disease detection and the rapid progression to chemoresistance have prevented appreciable improvement in the five year survival rate of patients with ovarian cancer.

Experimental models for human diseases are of crucial importance not only to understand the biological and genetic factors that influence the phenotypic characteristics of the disease but to utilize as a basis for developing rational intervention strategies. Ovarian cancer cell lines derived from ascites or primary ovarian tumors have been used extensively and can be very effective for studying the processes controlling growth regulation and chemosensitivity. Our limited knowledge of the initiating events of ovarian cancer has restricted the development of models in which the early pathogenic events for ovarian cancer can be studied. However, there are a few animal models that develop ovarian tumors spontaneously, and others where the manipulation of various reproductive factors or exposure to environmental toxins have been shown to promote ovarian tumorigenesis. Finally, the recent

identification of promoters that can drive gene expression in the ovarian surface epithelium is providing new opportunities for the generation of genetically engineered mouse models of ovarian cancer. Here we describe some of the models that have been developed to investigate ovarian cell transformation.

Spontaneous and Non-epithelial Ovarian Tumorigenesis

There are few animal models that develop ovarian tumors spontaneously. Hens maintained under intensive egg-laying conditions develop ovarian adenocarcinomas; however such tumors are uncommon in hens less than 2 years of age [1]. Ovarian tumors will also arise spontaneously with age in some strains of mice [2], and in Wistar and Sprague-Dawley rats [3,4]. These tumors show a wide variety of histologic sub-types, including tubular adenoma, adenocarcinoma, papillary cystadenoma, mesothelioma, granulosa cell tumor, and polycystic sex cord/stromal tumor. However, the low incidence and/or the length of time required for the appearance of tumors in all of these models render them poorly feasible for experimental studies of ovarian carcinogenesis.

Some strains of mice, including C3HeB/Fe and C3HeB/De, show a high incidence of spontaneously occurring granulosa cell tumors and tubular adenomas [5]. Strain HAN:NMRI develop spontaneous Sertoli cell-like tumors and (DBA × Ce)F1 hybrids have a high incidence granulosa cell tumors [5]. Granulosa cell tumors also appear spontaneously at 4–6 weeks of age in SWR/J and in SWR/Bm inbred strain mice, with a maximum incidence reached by 10 weeks [6]. In some SWXJ strains, granulosa cell tumors occur spontaneously, and in others granulosa tumors can only be induced by treatment with dehydroepiandrosterone [7].

Spontaneous germ cell tumors are less common, but have been reported in LT/Sv and related strains of mice. These mice have a high frequency of spontaneous ovarian teratomas arising from follicular oocytes that undergo parthenogenetic activation. In some strains, this defect appears to be associated with an arrest of the oocytes at metaphase of meiosis I [8]. Teratomas arising from parthenogenetic activation of oocytes also occur in c-*mos*-deficient oocytes, which fail to maintain meiotic arrest after oocyte maturation [9,10].

Mice generated to be deficient in the tumor suppressor gene Lats1 exhibit a lack of mammary gland development, infertility and growth retardation. Accompanying these defects are hyperplastic changes in the pituitary and decreased serum hormone levels. The reproductive hormone defects of Lats1-/- mice are reminiscent of isolated LH-hypogonadotropic hypogonadism and corpus luteum insufficiency in humans. Lats1-/- mice develop soft-tissue sarcomas and ovarian stromal cell tumors [11].

The Ovarian Surface Epithelium

Although ovarian cancer in humans can arise from any of the cell types found in the ovary, almost 90% are derived from the ovarian surface epithelium (OSE) [12]. The OSE covers the entire ovarian surface, and varies morphologically from simple squamous to cuboidal to low pseudostratified columnar [13,14]. Embryologically derived from the mesodermal epithelium of the gonadal ridges, OSE cells are continuous with the flattened mesothelium of the peritoneum [15] and are separated from the underlying stromal compartment of the ovary by a basement membrane. Immunohistochemical staining has shown that OSE cells express cytokeratin, desmoplakin, transforming growth factor-α (TGF-α) and receptors for estrogen, progesterone and epidermal growth factor (EGF) [16–20]. Despite their rather unremarkable appearance *in vivo*, it is believed that OSE cells actively participate in the ovulatory process. Studies in rabbits and sheep have shown that OSE release proteolytic enzymes that degrade the basement membrane and the underlying apical follicular wall, weakening the ovarian surface to the point of rupture [21]. The OSE cells directly over the point of rupture undergo apoptotic cell death before ovulation [22] and the wound created at the ovulatory site surface is repaired by rapid proliferation of OSE cells from the perimeter of the ruptured follicle [23]. The biology, endocrinology and pathology of the ovarian surface epithelium have recently been reviewed in detail [24].

Although the ovarian surface is generally smooth in early reproductive life, with aging the ovary becomes more convoluted. Invaginations of the epithelium result in crypts or gland-like structures that can become pinched off to form epithelial inclusion cysts within the underlying stromal compartment [25]. This may occur following the postovulatory proliferation of OSE, follicular attrition, and/or from inflammation caused by carcinogens or chemical irritants like talcum powder [26]. The incidence of inclusion cysts increases with advancing age and are common in postmenopausal women. Although generally benign in nature, these epithelial rearrangements are widely thought to be the potential origin of many epithelial cancers. The more frequent appearance of epithelial invaginations and inclusion cysts in women with hereditary risk of ovarian cancer has strengthened this hypothesis [27]. In addition, some microscopic borderline and malignant tumors have been observed to arise directly within these sites, and they are often associated with dysplasia in similar sites elsewhere in the same or contralateral ovary [28,29].

Xenografts of OSE Cells Transformed *in vitro*

OSE cells have been implicated as the cell of origin for the majority of ovarian cancers based primarily on histological and immunohistochemical analyses of patient samples, but several recent experimental models manipulating these cells *in vitro* have provided additional support for this concept. Primary culture of human OSE was first reported by Auersperg et al. in 1984 [30], and her group has since developed several *in vitro* models of ovarian epithelial carcinogenesis. Introduction of Kirsten murine sarcoma virus into rat OSE cells results in endometrioid tumors following subcutaneous or intraperitoneal injection into immunosuppressed rats [31]. Transfection of SV40 T antigen early genes induces immortalization of human OSE cells that delays, but does not prevent, the senescence that normally occurs after a few passages [32]. Introduction of E-cadherin into these T antigen-immortalized cells induces epithelial differentiation [33] and the cells formed transplantable, invasive adenocarcinomas when injected into SCID mice [34]. In contrast to T antigen-immortalized cells, introduction of the human papilloma virus E6 and E7 genes into human OSE cells results in the spontaneous progression from a benign to invasive phenotype [35].

Unlike human OSE, rat and mouse OSE do not senesce. Rat OSE cells that have spontaneously immortalized but are not tumorigenic (eg. ROSE 199 cells; [36]) have been used in a variety of experiments, including some to characterize the cellular features when SV40 T antigen or H-*ras* is introduced into immortalized cells and following the formation of tumors when these cells are xenografted into nude mice [37]. Repeated subculture of rat and mouse OSE cells to maintain continued proliferation results in spontaneous malignant transformation, as characterized by loss of contact inhibition, substrate-independent growth and the ability to form tumors in nude mice [38,39]. In a variation of the above *in vitro* transformation approaches, Orsulic and colleagues used the RCAS retroviral vector to introduce oncogenes into OSE cells from transgenic mice bearing the RCAS receptor TVA and the cells were evaluated for tumorigenicity by injection into immune-deficient or syngeneic animals [40]. The investigators found that p53 deficiency in combination with two oncogenes from among C-*MYC*, *K-RAS*, or *AKT* were required to achieve transformation.

While these models allow an evaluation of oncogenes whose activation may contribute to the development of epithelial ovarian cancer, this approach does not allow the investigation of the early events in ovarian tumorigenesis inherent in mice when the tumors arise *in situ*. However, the establishment of *in vitro* models of normal and transformed OSE cells has provided the opportunity to use molecular approaches such as microarray or suppression subtractive hybridization to identify differential gene expression patterns that can distinguish normal OSE and ovarian cancer cells [41,42]. These data will be useful for the elucidation of molecular events associated with OSE cell transformation.

Xenografts of Cancer Cells

Xenograft models, where ovarian cancer cells have been injected either subcutaneously or into the peritoneal cavity have been used extensively for the testing of novel therapeutics or modified regimens for administration of standard chemotherapeutic drugs [43–45]. Some mouse models take advantage of the presence of a bursa, a sac-like structure that envelops rodent ovaries. For decades, researchers have used the intra-bursal space for transplants of xenografted ovaries, or to facilitate direct exposure of the ovary to various factors. For the generation of mouse models of ovarian cancer, the injection of ovarian cancer cells into the intra-bursal space results in tumor formation that can perhaps be viewed as more physiological (Figure 1), as the cancer cells are placed directly in the environment where ovarian tumors normally arise [46].

Reproductive Factors and Ovarian Tumorigenesis

Unlike most other cancers, the series of events involved in the initiation, progression and metastasis of ovarian cancer is not yet established. It is not clear if malignancies arise from benign or borderline tumors or if they develop *de novo* from the surface epithelium or inclusion cysts, as there is evidence for both [47]. The incidence of ovarian cancer climbs dramatically in women around the age at which they reach menopause. The reason for this is not clear, but two of the major changes associated with menopause form the foundation for hypotheses regarding the origin of ovarian tumors: 1) the depletion of oocytes or germ cells, which is the underlying cause of menopause, and 2) a significant increase in the pituitary's production of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), that arises as a consequence of the reduced follicular estrogen levels. In addition to the loss of germ cells and the associated alterations in hormone levels which normally occur at menopause, there are a number of non-menopausal factors that have been shown to have physiological relevance in epithelial ovarian tumorigenesis, including ovulation. Each of these will be discussed in the context of the animal models that have resulted from the experimental manipulations of these factors.

Ovulation

The "incessant ovulation hypothesis" proposes that continuous ovulation, with its successive rounds of surface rupture and OSE cell mitosis to repair the wound, renders the cells susceptible to malignant transformation [48].

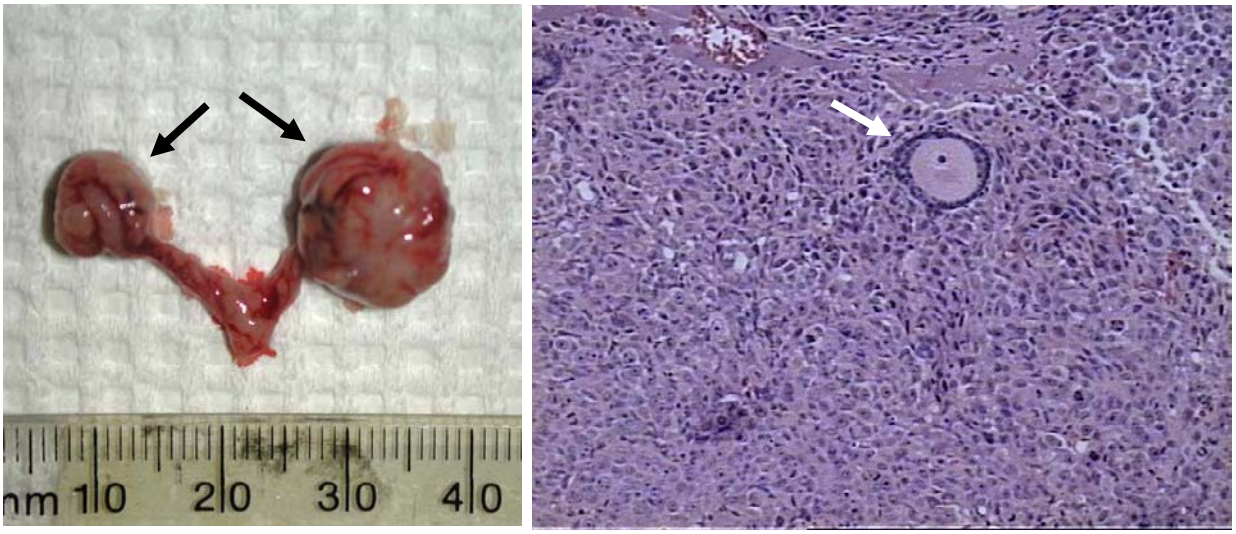

Figure 1
Development of ovarian tumors following injection of ES-2 ovarian cancer cells under the bursal membrane of nude mouse ovaries. Left figure- Proliferating cancer cells invade the normal tissue and increase the ovarian mass to diameters > 10-fold in size (indicated by arrows). Right figure- A single follicle containing a growing oocyte, indicated by an arrow, is clearly visible in the mass of tumor tissue.

Anecdotal support for this hypothesis comes from the observation that intensive egg-laying domestic hens frequently develop peritoneal carcinomata that is presumably of ovarian origin [1]. Epidemiological studies indicate that circumstances that decrease the number of ovulations, i.e., pregnancy, oral contraceptive usage, duration of lactation and early menopause, all substantially reduce the risk of ovarian cancer [49,50].

Inherent in the incessant ovulation hypothesis for ovarian cancer risk is the premise that repetitive damage of the OSE at ovulation and/or the subsequent mitotic repair following ovulation increases the risk of developing ovarian cancer. Experimental evidence to support the susceptibility of OSE cells to mutagenic events during mitosis is provided by studies showing that primary cultures of normal rat and mouse OSE cells which have been repeatedly subcultured to maintain continued proliferation acquire features associated with malignant transformation, including loss of contact inhibition, substrate-independent growth and the ability to form tumors in nude mice [38,39].

The risk generated by incessant ovulation may also be associated with the formation of epithelial cell-lined inclusion cysts that are frequently found in the ovarian stroma of perimenopausal women. As noted above, these inclusion cysts may form as a result of the process of ovulation and the pinching off of deep clefts [47]. In mice, the lifetime total number of ovulations is associated with a marked increase in OSE invagination and stratification [51], although the incidence of inclusion cysts was more related to age than to number of ovulations. Therefore, unlike in humans, an association between number of ovulations and ovarian cancer risk has not been demonstrated in rodents.

Gonadotropins

An alternative, but not mutually exclusive, hypothesis for the mechanism of ovarian carcinogenesis proposes that the development of ovarian tumors is related to excessive gonadotropin production associated with the onset of menopause or premature ovarian failure [52]. The median age for epithelial ovarian cancer is 60–65 years, with only 10–15% of the tumors appearing in premenopausal women [53]. Serum FSH and LH levels reach their peak during perimenopausal and postmenopausal years and remain elevated thereafter [54]. High circulating levels of pituitary gonadotropins may increase the risk of ovarian cancer by stimulating the growth of ovarian epithelial

cells, since normal human OSE cells and epithelial inclusions have been found to express receptors for FSH [55] and LH/hCG [56]. Enhanced cell proliferation in response to FSH and/or LH/hCG has been reported for primary cultures of rabbit [57], mouse [58] and human [56] OSE cells. Schiffenbauer and colleagues [59] found that human epithelial ovarian cancers progressed faster in ovariectomized mice due to elevated FSH and LH levels, which promoted increased vascular endothelial growth factor expression and tumor neovascularization.

The gonadotropin theory of ovarian tumorigenesis suggests that elevated gonadotropin concentrations contribute to the development of ovarian tumors. This theory is based on the initial observation of Biskind and Biskind in 1944 [60] who reported that transplantation of ovaries into the splenic pulp of adult rats led to the development of ovarian tumors. The tumorigenesis was attributed to inactivation of estrogen in the liver, and the consequent elevation of gonadotropin levels due to the lack of steroid feedback on the pituitary. Several transgenic or knockout animal models in which gonadotropin levels are elevated also result in ovarian tumorigenesis. For example, when inhibin, the ovarian protein that inhibits the production of FSH, is made deficient in mice, gonadal stromal tumors arise [61]. Transgenic mice generated to have chronic LH hypersecretion develop granulosa cell tumors or luteomas, depending on the background strain [62,63]. Mice with disruption of the FSH receptor are acyclic and sterile, with very small, underdeveloped ovaries; they exhibit hypergonadotropic-hypogonadism with high levels of circulating FSH and LH similar to the postmenopausal state in women. By 12 months, more than 92% of these animals developed various kinds of ovarian pathology, including neoplasms of sex cord-stromal type as well as cysts, suggesting that FSH receptor insensitivity in the face of prolonged elevated levels of gonadotropins may be contributing to the development of ovarian granulosa or stromal tumors [64]. None of the animal models with targeted manipulation of gonadotropin secretion or action appear to promote ovarian epithelial tumorigenesis.

Steroid hormones

In the developing fetal ovary, marked OSE cell proliferation occurs at 16 to 20 weeks of gestation, coincident with the appearance of steroid-producing cells in the ovarian cortex [65]. Adult human OSE cells express receptors for estrogen, progesterone and androgens [66,67], and human OSE cell proliferation can be stimulated by androgens [68]. In contrast, human OSE cells in culture are reportedly unaffected by estradiol or progesterone [66], which would suggest that these steroid hormones do not have a significant role in ovarian tumorigenesis. However, a recent study has found that menopausal women who have taken hormone replacement therapy using estrogen only are at an increased risk of ovarian cancer [69]. In animals, continuous exposure to estradiol stimulates sheep OSE cell proliferation [70], while in guinea pigs and rabbits, it results in the formation of a papillary ovarian surface resembling human serous neoplasms of low malignant potential [71,72]. The mechanisms by which estrogen may contribute to ovarian cancer risk is unknown, but could be direct action on the OSE cells, or may be indirect, as estrogen reduces GnRH receptor expression in both OSE and ovarian cancer cells, thereby suppressing the growth inhibitory effects of GnRH [73]. Estrogen also modulates levels of hepatocyte growth factor which stimulates OSE cell growth [74].

A number of studies, largely epidemiological, provide support for the hypothesis that androgens are involved in ovarian carcinogenesis. Over 80% of tumors express AR [75] and an increased risk of ovarian cancer was found in women with elevated circulating levels of androgens [76]. Testosterone-stimulated growth of OSE cells in guinea pigs caused the formation of benign cysts, small adenomas in the ovarian parenchyma, and papillomas on the ovarian surface [77]. Androgens may promote ovarian tumorigenesis in part by decreasing TGF-β receptor levels, thereby allowing ovarian cancer cells to escape TGF-β growth inhibition [78].

Germ cell deficiency/depletion

Aging and hereditary risk are associated with a more frequent incidence of epithelial invaginations and inclusion cysts, putative preneoplastic precursor lesions, but the underlying mechanisms for these epithelial-stromal rearrangements are unknown. OSE cell hyperplasia with stromal invasion has been reported in a diverse array of experimental situations, all of them involving loss of germ cells and consequent failure of follicle development. For example, mutations at the *W* (*Kit*) or *Sl* (*Kitl*) loci result in sterility by preventing the normal proliferation and migration of germ cells during fetal development [79]. Germ cell deficiency *in vivo*, as is found in W^x/W^v mice, results in bilateral ovarian tubular adenomas in more than 95% of the animals by 5 months of age [80,81]. The tumors arise from interstitial cell hyperplasia, with proliferation and invasion of the ovarian surface epithelium into the stromal compartment of the ovary. Invasive epithelial tubules are also found in Sl/Sl^t germ cell deficient mice by 7 months of age [82], and mice heterozygous for the Sl^d mutation, which carries a splicing defect, develop papillary structures and epithelial invaginations (Figure 2), similar to that seen in women [26]. Likewise, female mice homozygous for the germ cell deficient (*gcd*) mutation enter reproductive senescence prematurely due to a dearth of germ cells. By one year of age, 56% of homozygotes have developed ovarian tubulostromal adenomas while wild-type littermates are phenotypically normal [83].

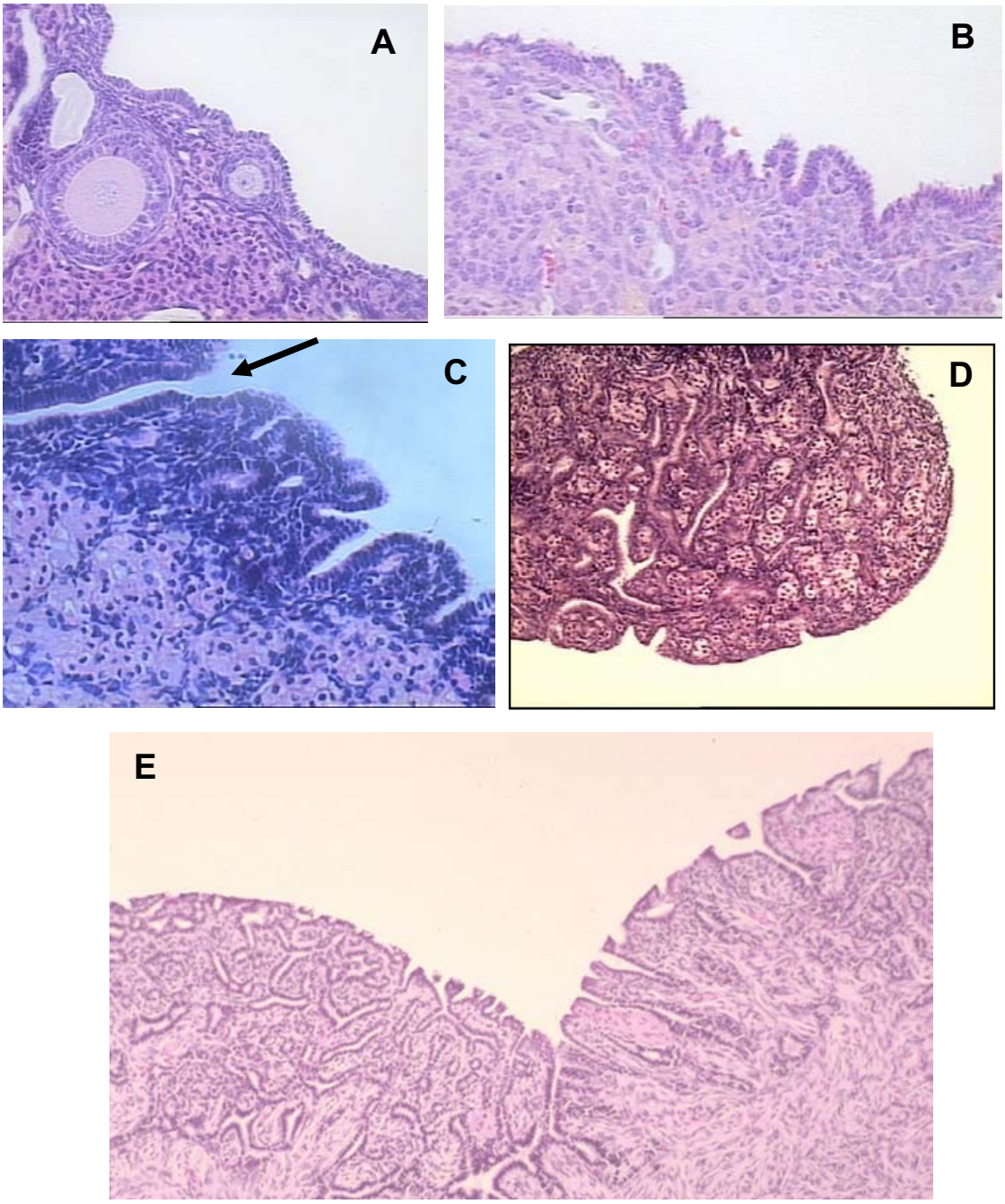

Figure 2
Morphology of the ovarian surface epithelium in wild-type (A; 12 months), *Sl*^{d} heterozygous (B, C; 12 months) and homozygous (D; 6 months) mice. Ovaries from wild-type mice contain developing follicles and a covering layer of columnar OSE. In 12-month-old *Sl*^{d} heterozygous mice, there is a depletion of follicles, and the ovarian surface has become very convoluted (B), with this papillary surface sometimes leading to deep invaginations, as indicated by the arrow (C). By 6 months of age, the ovaries of homozygous *Sl*^{d} mice are completely abnormal, with no recognizable ovarian structures, and are composed primarily of invasive epithelial tubules. (E) Human ovarian papillomatosis, for comparison.

Therefore, it appears that oocyte depletion is associated with formation of epithelial structures that resemble the preneoplastic lesions in human ovaries.

Experimental ovarian tumorigenesis has been investigated in inbred and hybrid strains of mice and induced by a diversity of mechanisms including X-irradiation, oocytotoxic xenobiotic chemicals, ovarian grafting to ectopic or orthotopic sites, neonatal thymectomy, genetic defects reducing germ cell populations, and aging [reviewed in [84]]. While germ cell deficiency seems to be a required element for the development of epithelium-derived adenomas, the mechanisms by which germ cell loss contributes to tumorigenesis in these models remain unclear. Ovarian follicles do not develop in the absence of oocytes, indicating that the oocyte directs the development of follicles. Pathogenetic factors that prematurely destroy or diminish the numbers of germ cells lead to failure in follicle development and a resulting decrease in sex steroid hormone secretion (notably estradiol) leading to a compensatory over-production of pituitary gonadotropins, which places the ovary at an increased risk to develop tumors. Therefore oocyte depletion, similar to that which occurs naturally by the time of menopause, may be a contributing factor to the oncogenic behavior of the surface epithelial cells.

The intense proliferation of OSE and stromal (interstitial) cells with the development of unique tubular adenomas in response to sterility seems to require both the lack of germ cells/follicles and the increased production of gonadotropins. Elevated gonadotropins alone resulted in granulosa cell tumors or luteomas [62,63]. Oocyte destruction by gamma irradiation in hypogonadal mice deficient in gonadotropins did not result in the development of tubular adenomas [85]. Similarly, the experimental suppression of gonadotropin levels in W^x/W^v mice was sufficient to prevent the development of ovarian tubular adenomas from the surface epithelium [86], suggesting that both oocyte loss/destruction and elevated gonadotropins are necessary for epithelial tumorigenesis.

Environmental Carcinogens

Although the more established hypotheses that have been proposed to explain increased risk of developing ovarian cancer are related to the number of ovulations or to increased hormone levels, there are additional risk factors that have been identified, including a number of environmental carcinogens. While these factors have been reported to have effects on the ovarian surface epithelium, they are usually also associated with follicular destruction and/or ovotoxicity, so indirect actions due to altered gonadotropin levels cannot be eliminated. Use of perineal talc has been identified as a risk factor, possibly due to its ability to ascend the genital tract and affect the ovarian surface [87]. Indeed, direct exposure of rat ovaries to talc results in focal areas of papillary change in the ovarian surface epithelium, as well as ovarian cysts [88]. Exposure of rhesus and cynomolgus monkeys to the environmental pollutant, hexachlorobenzene results in both reproductive failure and notable alterations in the size, shape and degree of stratification of the OSE cell layer [89]. More recent studies have shown that the insecticide methoxychlor increases both the height of the OSE cell layer and the percentage of atretic follicles in exposed mice [90]. In rodent studies, ovarian toxicity and/or carcinogenicity has been documented for at least eight chemicals that result in follicular necrosis, tubular hyperplasia, granulosa cell tumors and benign mixed tumors [91,92]. N-ethyl-N-nitrosourea administered to rats intraperitoneally or transplacentally increases the incidence of ovarian tubular adenomas [93]. The mechanisms by which these environmental carcinogens enhance the risk of ovarian tumors remain unexplored.

Transgenics and Targeted Approaches to Transform the Ovarian Epithelium

The ideal model to investigate the pathogenic events associated with early ovarian tumorigenesis would be a mouse model in which the tumor arises directly from the OSE cells. This model would differ from current xenograft models in that transgenic mice with defined genetic lesions could be studied at various stages as they inevitably develop ovarian cancer *in situ*. In addition, the development of a genetic model would permit the direct testing of oncogenes and tumor suppressors for their contribution to the initiation and progression of overt malignancies in the mouse ovary. Finally, a number of different factors could be altered such as the genetic background of the mouse strain, the frequency of ovulation and the levels of various hormones to determine their impact on the development of tumors in the susceptible transgenic mouse line.

One approach to alter gene expression directly in the OSE cells would be to take advantage of the fact that these cells readily take up and express genes delivered by intra-bursal injection of adenoviruses [94,95]. This method has the potential advantage of mimicking somatic mutations that contribute to early ovarian tumorigenesis. One recent report used intra-bursal adenovirus delivery and Cre-loxP mediated gene inactivation to render OSE cells deficient in two key tumor suppressor genes: p53 and Rb [95]. The p53 tumor suppressor gene is the most frequently mutated gene in human neoplasms. Mutations and/or over-expression of p53 have been described in 26–62% of ovarian cancers, particularly serous ovarian carcinomas [reviewed in [96]]. Aberrations in the Rb pathway have been reported [97]; however, direct evidence for their contribution to ovarian epithelial tumorigenesis is lacking. In

this model, recombinant adenovirus expressing Cre was injected under the ovarian bursal membrane of double transgenic mice bearing floxed copies of *p53* and *Rb*. Concurrent inactivation of p53 and Rb was sufficient for reproducible induction of ovarian epithelial carcinogenesis in mice homozygous for the conditional alleles. While less than 15% of mice with inactivation of either *Rb* or *p53* developed tumors, 33 of 34 mice with deficiencies in both genes succumbed to their ovarian cancers at a median of 227 days, with 24% having abdominal ascites.

The major impediment to the development of transgenic models of ovarian cancer is the lack of specific promoters able to direct gene expression to OSE cells. Previous models of ovarian cancer have resulted in granulosa cell tumors using promoters, such as inhibin-alpha subunit promoter, that are active in this cell type to drive the expression of the large T antigen of SV40 [98,99]. Recent studies have identified two other promoters that may prove to be useful for the generation of transgenic models of ovarian cancer. The Ovarian Specific Promoter (OSP-1) was developed from a retrovirus-like element specifically expressed in the rat ovary. The promoter drives gene expression specifically in normal and neoplastic ovarian epithelial cells [100] and expression of *lacZ* driven by OSP-1 in transgenic mice was restricted to the ovary as determined by X-gal staining of multiple organs [101]. Immunohistochemical detection of β-galactosidase showed *lacZ* expression mainly in the granulosa cells and ovarian surface epithelial cells. However, transgenic mice in which OSP-1 drives the expression of the early region of SV40 virus developed tumors in a variety of tissues, including unilateral granulosa cell tumors in two of three female founder mice. Thus, although transcription from the OSP-1 promoter occurs predominantly in the ovary, this promoter is sufficiently "leaky" in cells in other tissues to permit their tumorigenic conversion by SV40 TAg.

The first transgenic model of epithelial ovarian cancer was recently reported and used the upstream region of the Mullerian inhibitory substance type II receptor (*MISIIR*) gene to drive tissue-specific expression [102]. MISIIR is a single transmembrane serine/threonine kinase that shares homology with the TGFβ-receptor [103,104]. Expression of *MISIIR* has been reported to be restricted to mesenchymal cells surrounding the Mullerian duct during embryogenesis, tubular and follicular structures of fetal gonads, Sertoli and Leydig cells of adult testis, and granulosa cells of adult ovary [103,105,106]. More recently, expression of *MISIIR* in established human ovarian cancer cell lines as well as cell lines derived from the ascites of patients with ovarian carcinomas has been demonstrated [107]. Transgenic mice in which the 5' upstream regulatory sequences of the mouse *MISIIR* gene were used to target expression of the SV40 *TAg* specifically to the epithelium of the female mouse reproductive tract, including the OSE, developed ovarian carcinomas with metastatic spread to peritoneal organs by 3 months of age. Female transgenic mice developed bilateral ovarian tumors in ~50% percent of cases. Histologically, these tumors were poorly differentiated carcinomas with occasional cysts and papillary structures present at the surface of the ovary. These tumors disseminated intraperitoneally, invaded the omentum and formed ascites in a manner that resembles human ovarian carcinomas. The demonstration that the *MISIIR* promoter can be used successfully to drive gynecological tissue-specific transgene expression in mice and that this often results in the formation of ovarian carcinoma offers very promising opportunities for testing the efficacy of chemotherapeutic and chemopreventive agents in a heritable model of epithelial ovarian cancer.

Conclusions

The two most pressing problems in the management of ovarian cancer are the lack of adequate diagnostic or screening strategies, and the recurrence of disease that is often chemoresistant. In part, the deficiency in diagnostic tools is due to the lack of markers for the detection of preneoplastic or early neoplastic changes in the OSE cells. The generation of animal models in which OSE cells undergo neoplastic transformation *in vivo* will provide much-needed opportunities to investigate the cellular and molecular changes associated with the initiation of OSE cell transformation, as well as to provide models in which prevention, diagnostic, screening and therapeutic strategies can be developed.

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